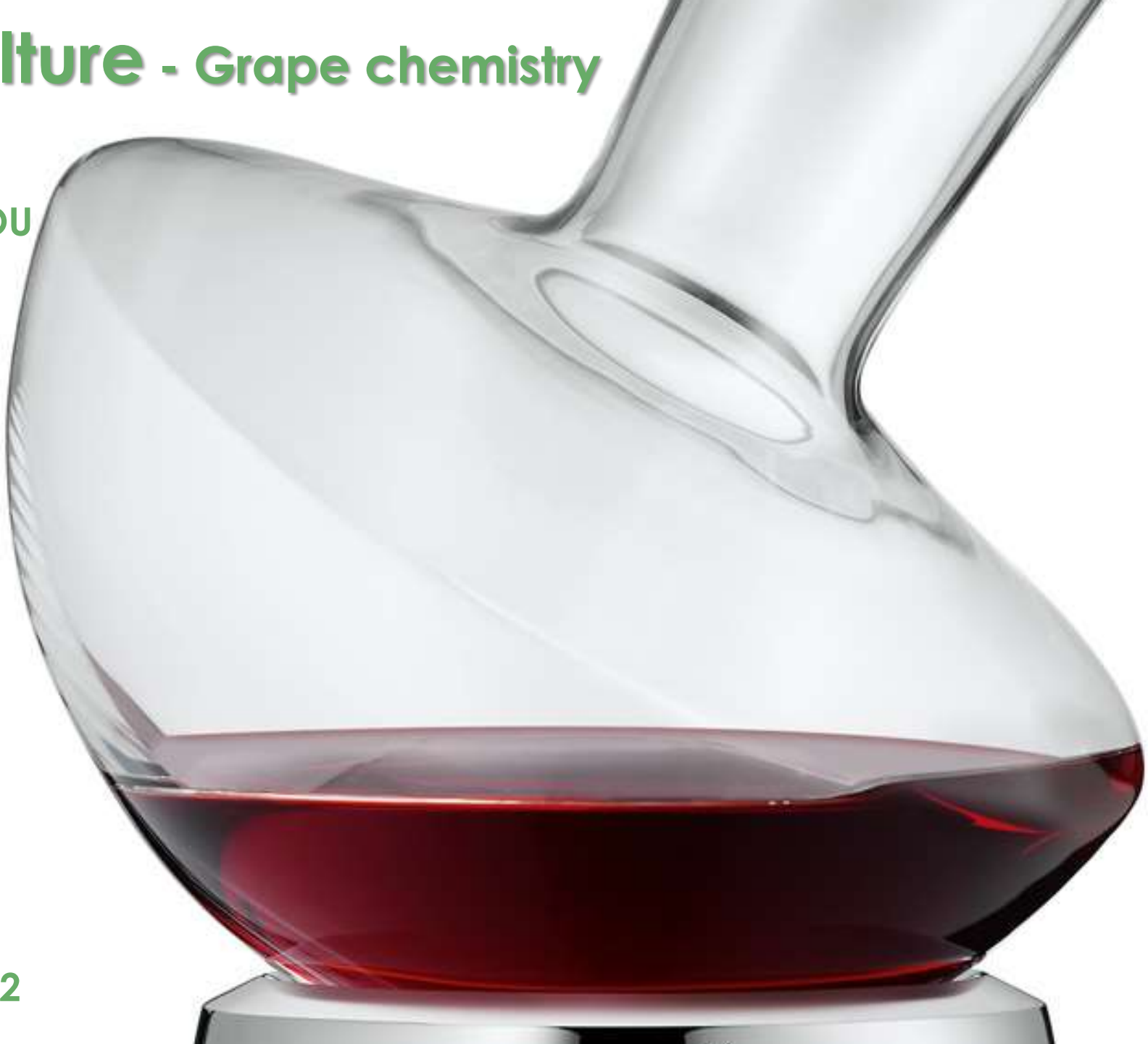
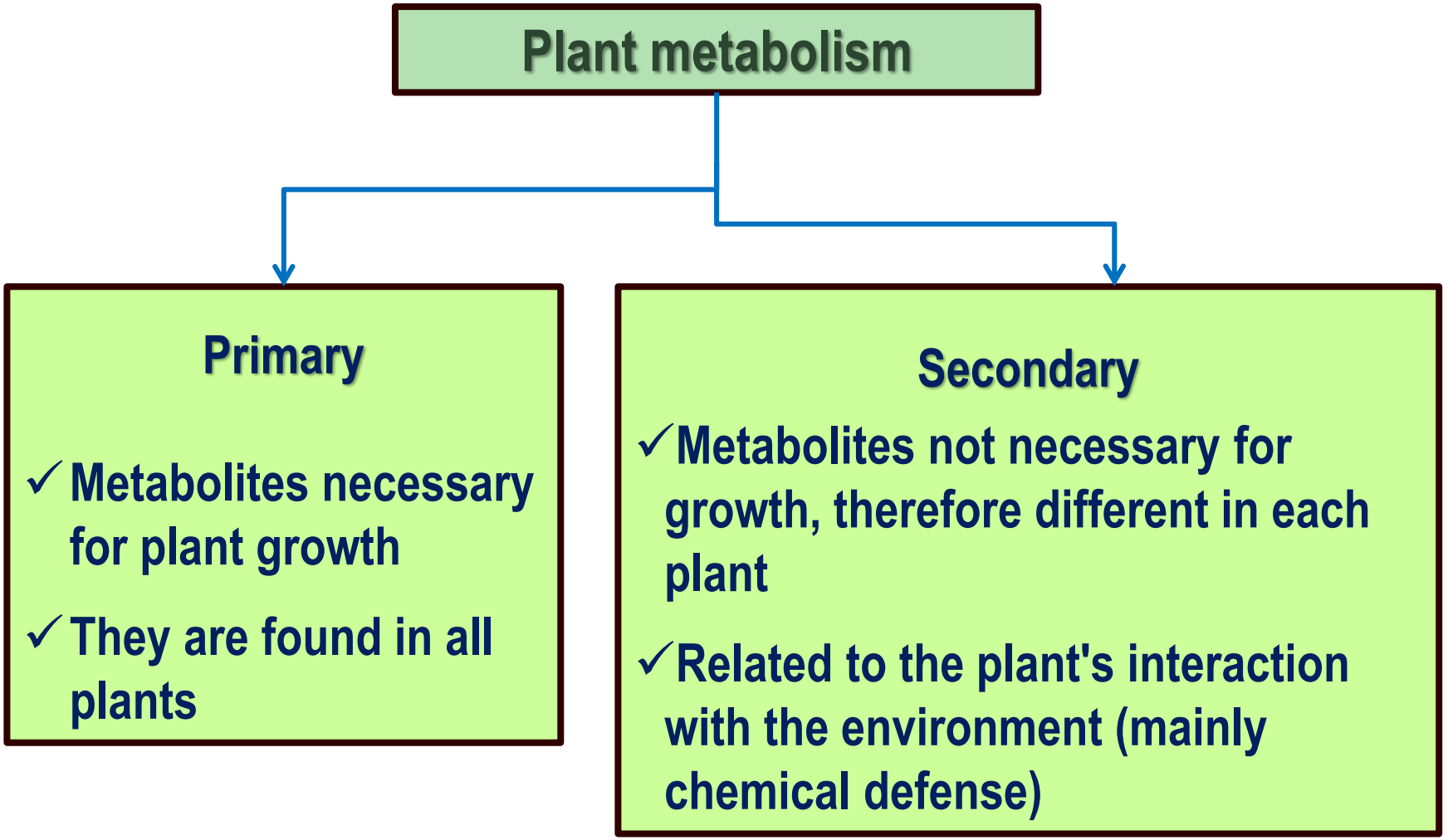
Viticulture - Grape chemistry

ARGYRO
BEKATOROU

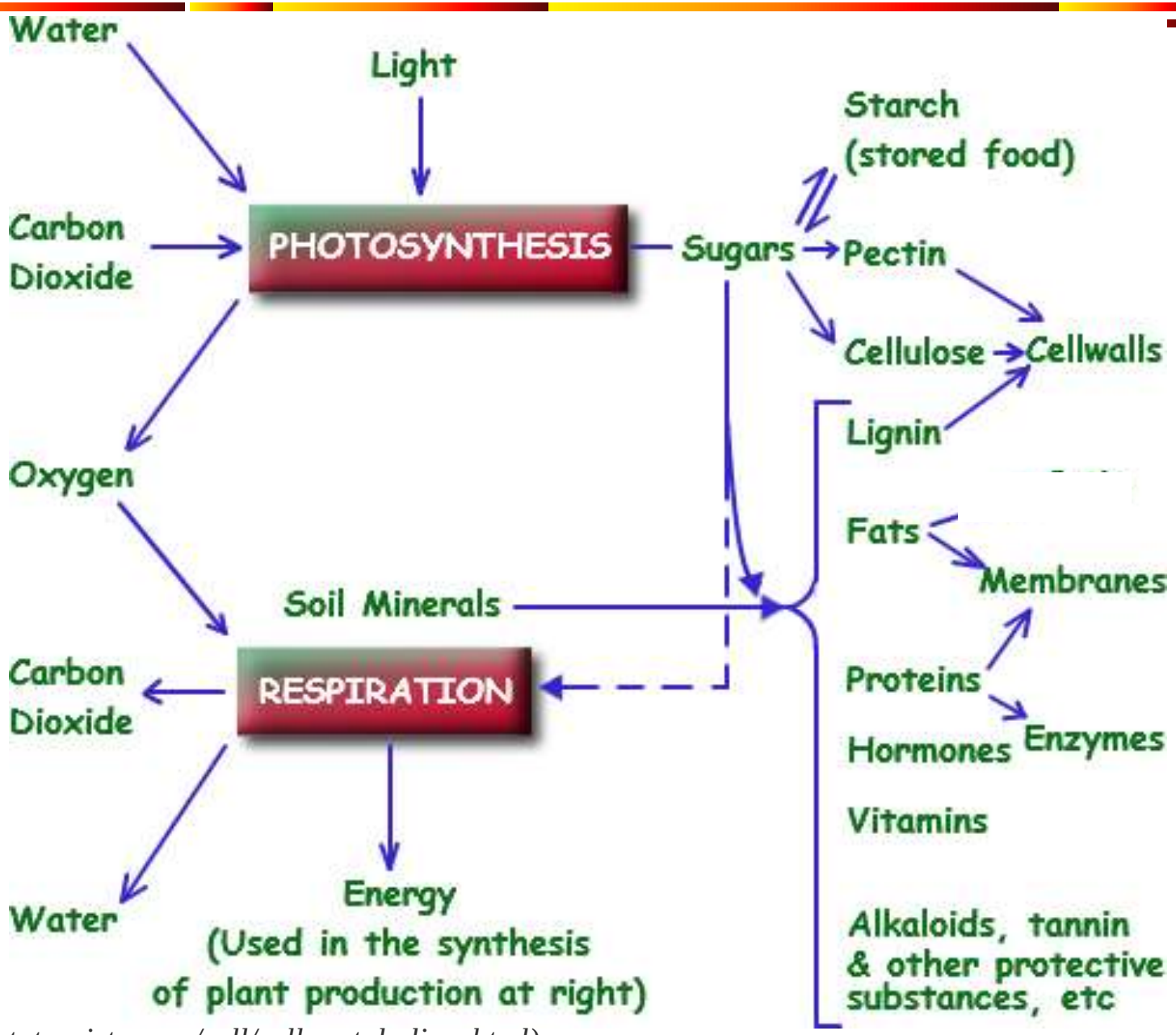


Patras 2022

Plant metabolism



Plant metabolism



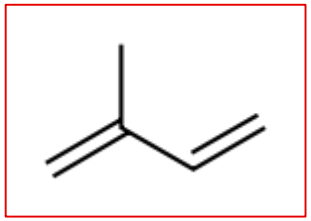
(http://biology.tutorvista.com/cell/cell-metabolism.html)

Plant metabolism

!!!

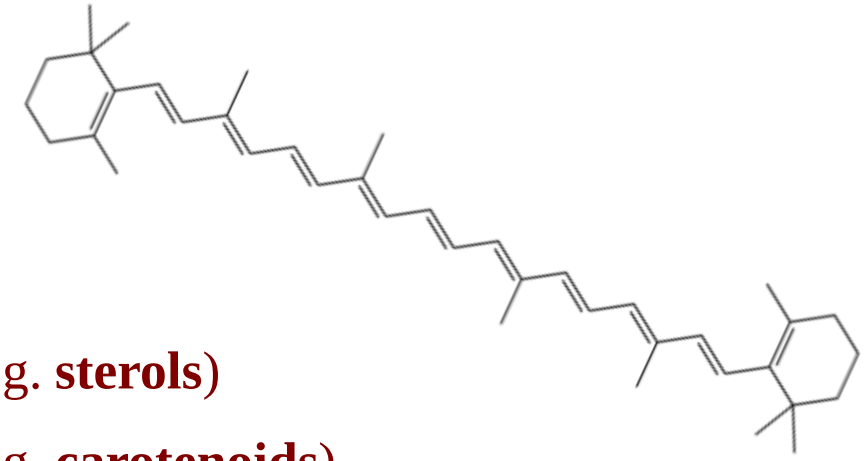
Secondary metabolites (recorded >190.000)

- **Terpenoids**



isoprene

- **Isoprene derivatives** (2-methyl-1,3-butadiene)
- Hydrocarbons but also oxygenated derivatives
- Straight chain & circular structures
- Depending on the isoprene units, they are divided into:
  - ✓ **Hemiterpenes** (**1** isoprene unit, 5 C atoms)
  - ✓ **Monoterpenes** (**2** isopr., 10 C)
  - ✓ **Sesquiterpenes** (**3** isopr., 15 C)
  - ✓ **Diterpenes** (**4** isopr., 20 C)
  - ✓ **Sesterterpenes** (**5** isopr., 25 C)
  - ✓ **Triterpenes** (**6** isopr., 30 C) (e.g. **sterols**)
  - ✓ **Tetraterpenes** (**7** isopr., 40 C) (e.g. **carotenoids**)
  - ✓ **Polyterpenes** (more C atoms)



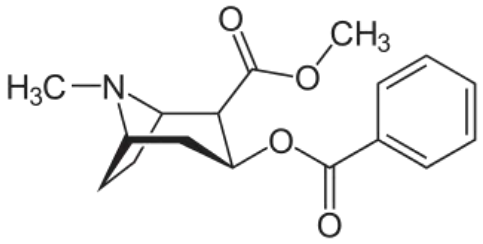
β-carotene

https://en.wikipedia.org

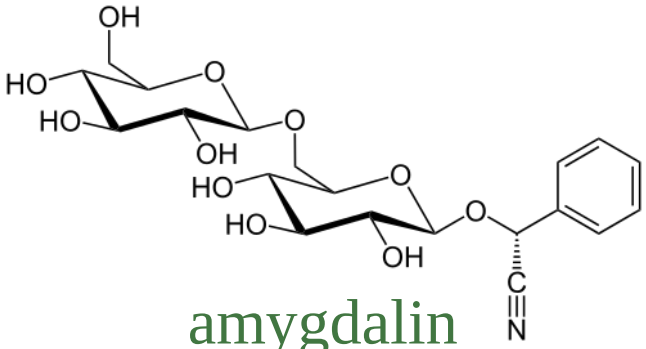
Plant metabolism

Secondary metabolites (recorded >190.000)

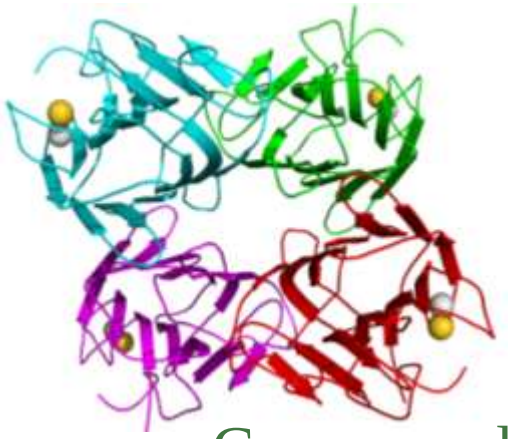
- **Phenolics**
- **Nitrogen compounds**
  - ✓ **Alkaloids** (cocaine, caffeine, morphine, quinine, nicotine, atropine)
  - ✓ **Amino acids not found in proteins**
  - ✓ **Amines**
  - ✓ **Cyanogenioc glucosides** (amygdalin)
  - ✓ **Alkamides**
  - ✓ **Lectins** (Concanavalin A)
  - ✓ **Peptides, polypeptides, etc.**



cocaine



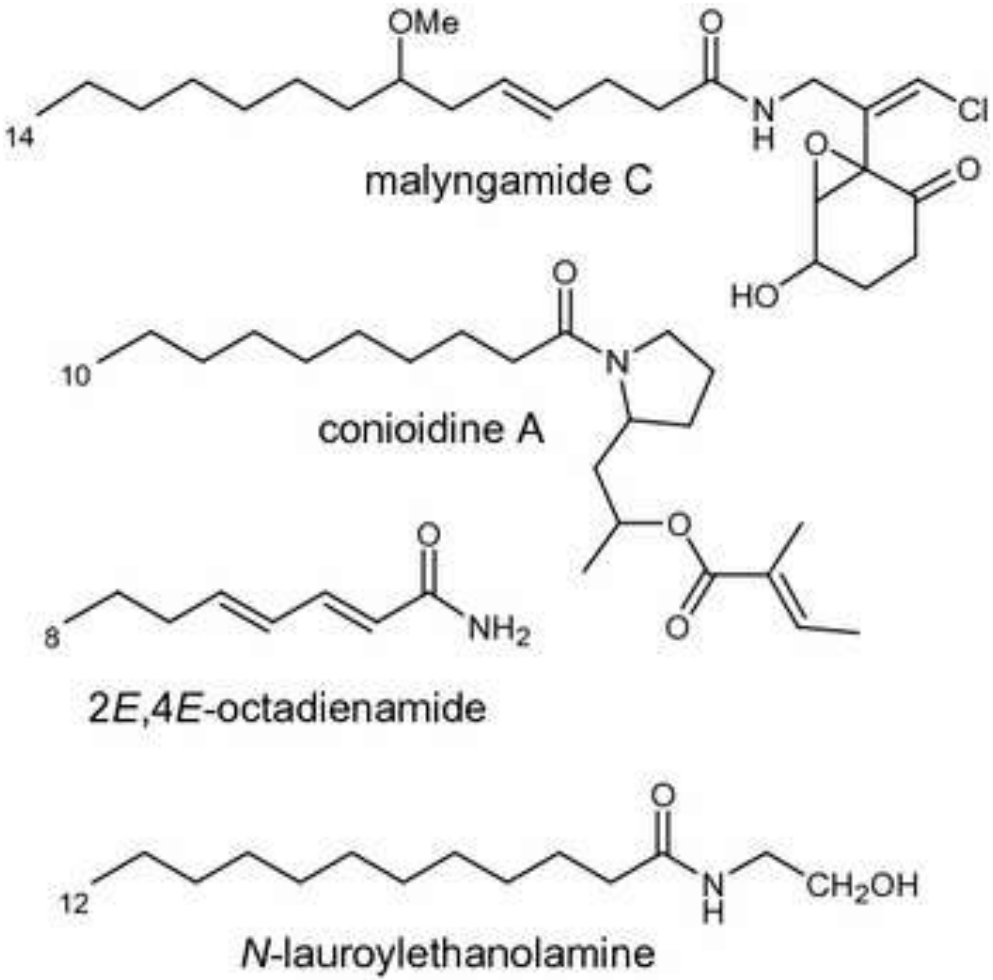
amygdalin


Concanavalin A

Plant metabolism

Secondary metabolites (recorded >190.000)

- ✓ **Alkamides**
  - Natural products formed by the linear chain bonding of unsaturated, aliphatic acids to various amines through an amide bond.
  - > 300 derivatives are known from 8 plant families, consisting of various combinations of 200 acids with 23 amines



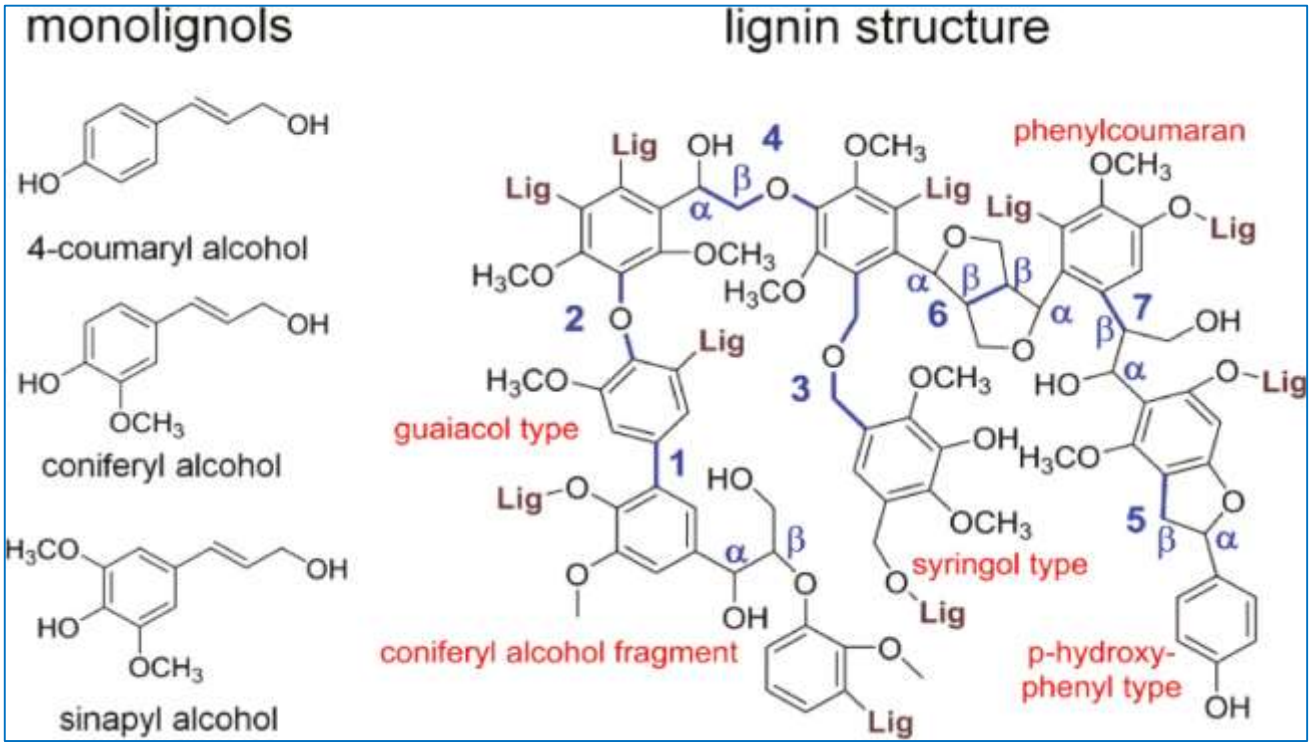
Plant metabolism

Secondary metabolites (recorded >190.000)

- **Phenolics**

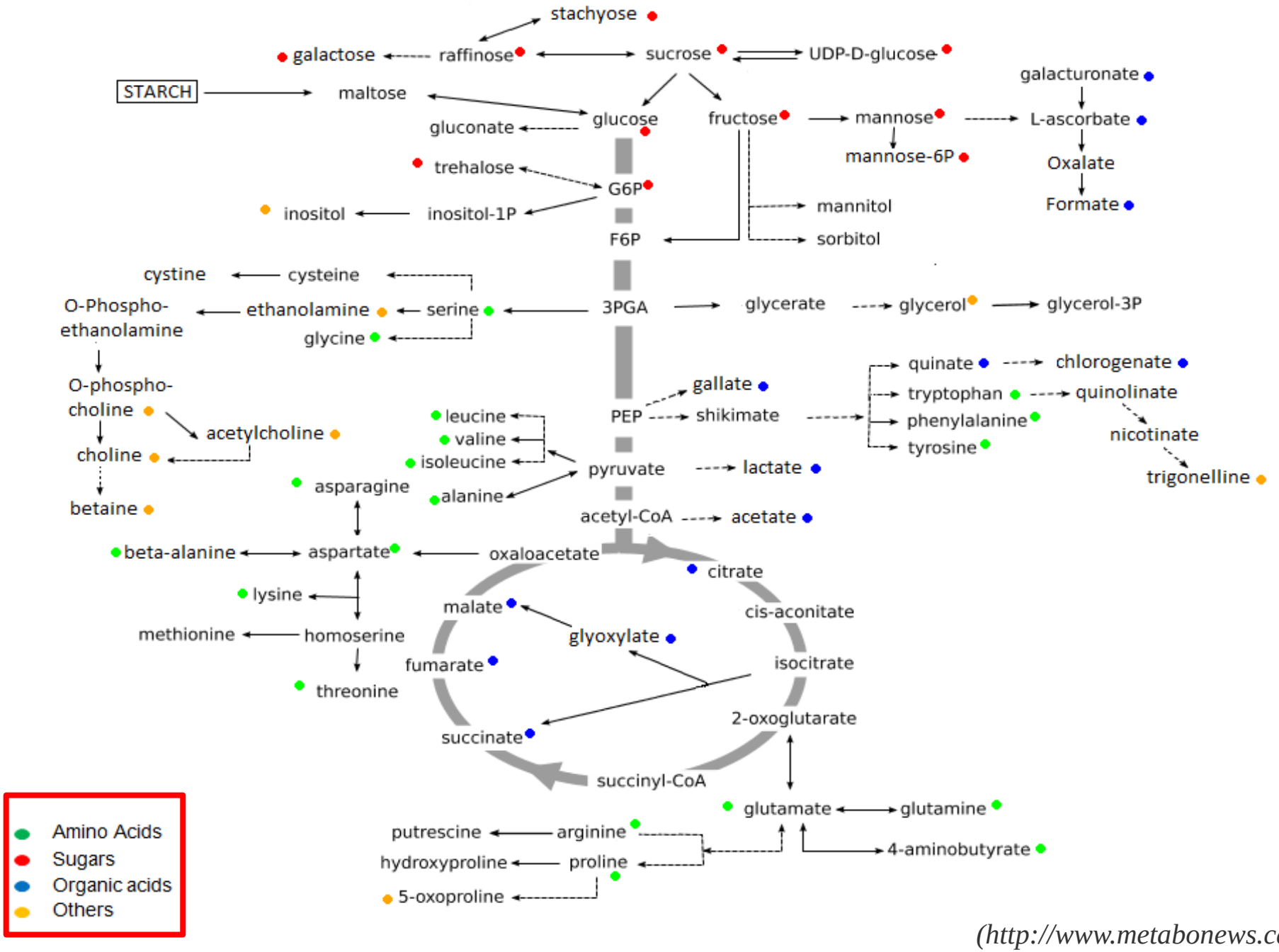
✓ **Lignin**:

- Highly branched and amorphous biomacromolecule (MW 1000-20,000)



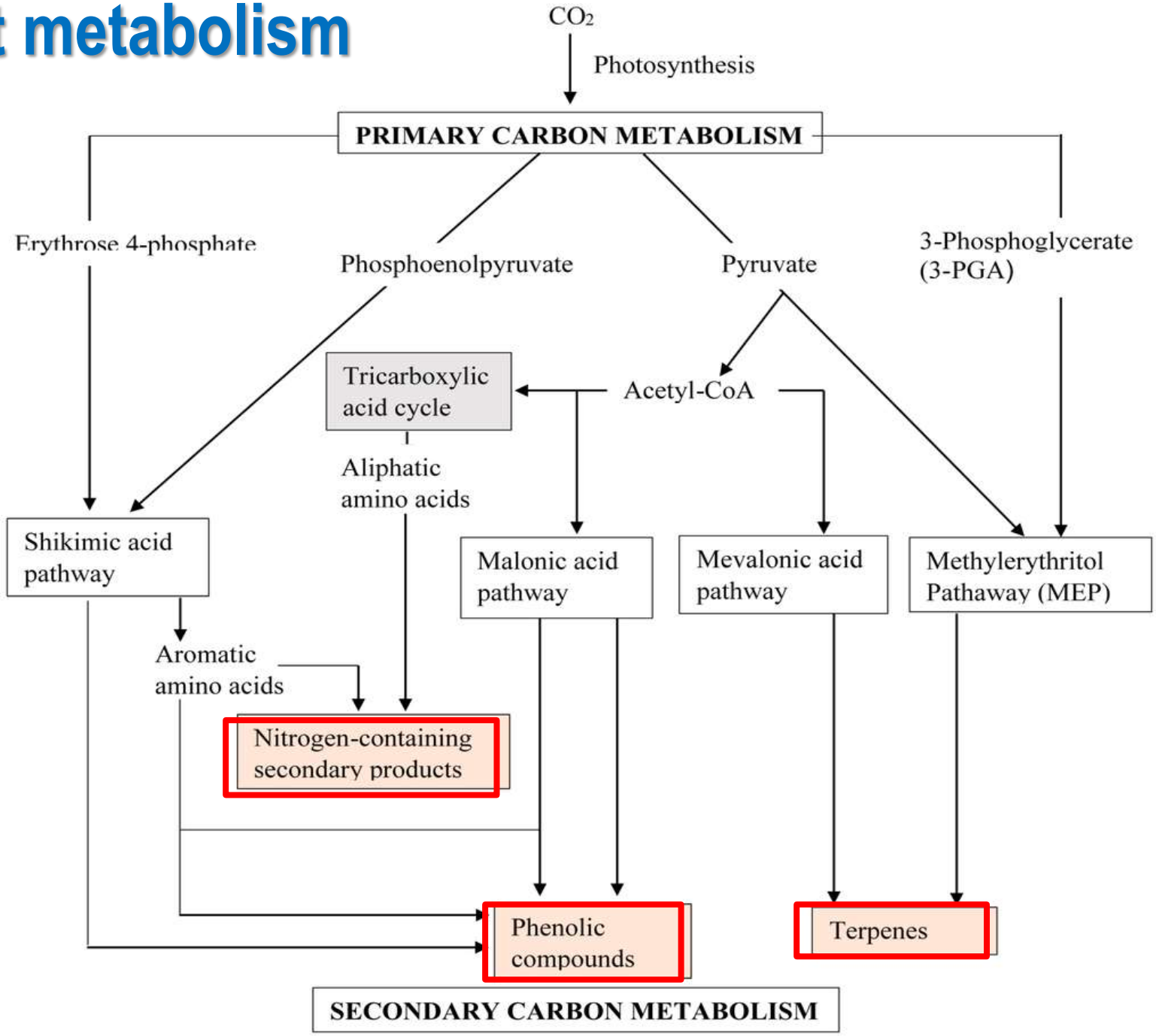
- 3 basic phenylpropanol monomers (**lignols**): **coniferyl alcohol** (4-hydroxy-3-methoxyphenylpropane), **sinapyl alcohol** (3,5-dimethoxy-4-hydroxyphenylpropane), and **paracoumaryl alcohol** (4-hydroxyphenylpropane)

A simplified map of plant metabolism (primary & secondary), with the identified compounds spotlighted (57/77).

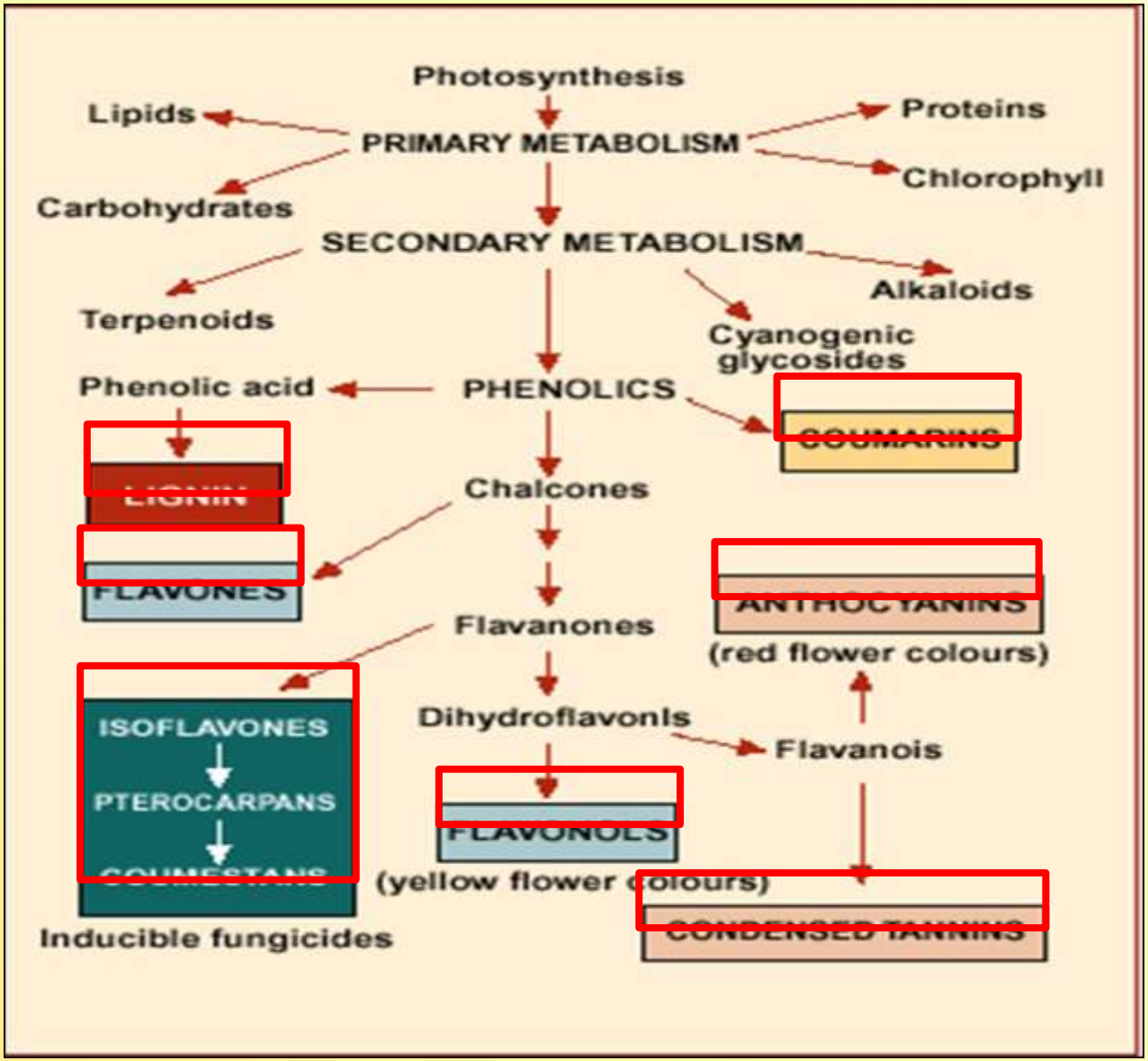


(http://www.metabonews.ca)

Plant metabolism



Plant metabolism

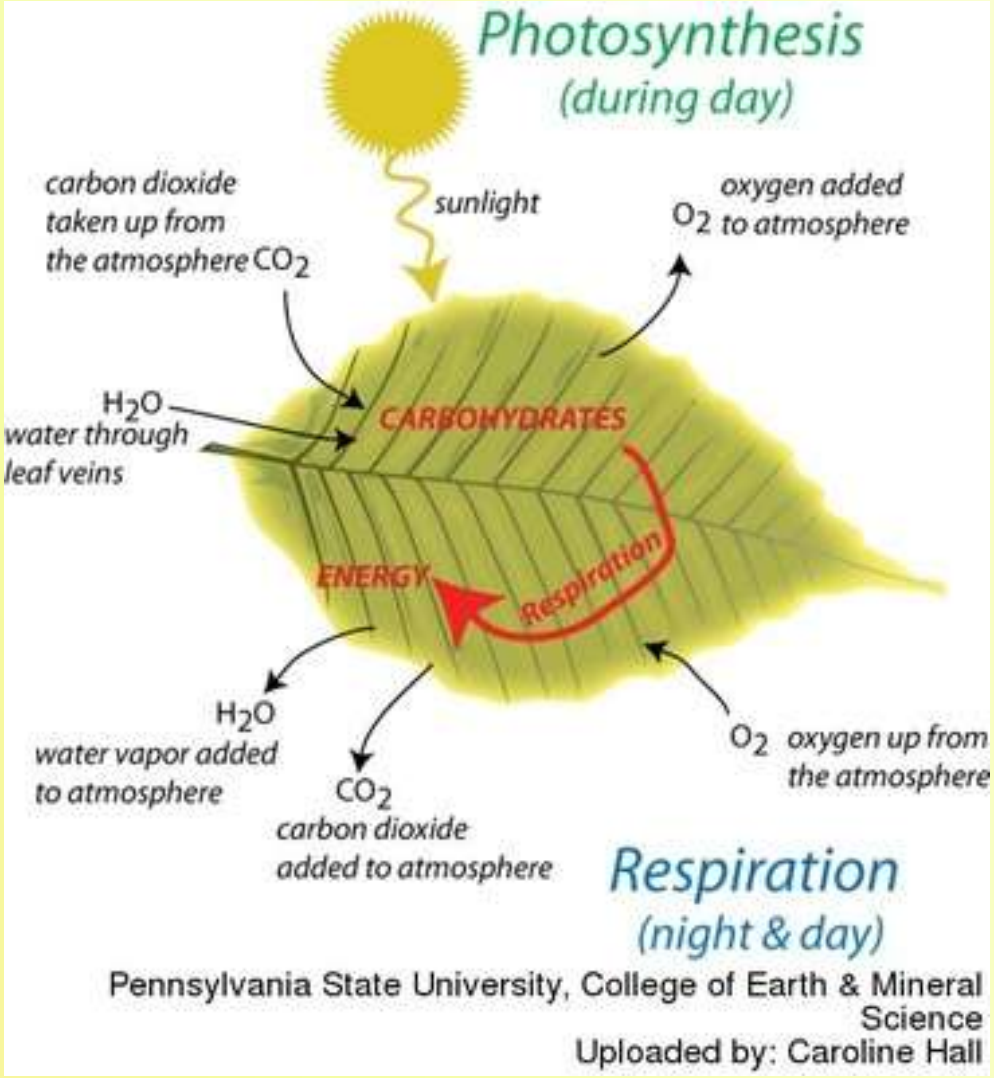


http://www.science.marshall.edu/valluri/HFB.htm

Viticulture – Grape chemistry

Vine leaf

- Leaf functions
  - Transpiration of water from the atmosphere
  - Carbon dioxide fixation – photosynthesis
  - Synthesis of organic compounds



https://guides.itsi.concord.org/photosynthesis-teacher-guide.html

Grape berry: evolution of maturation

- **First period:**

Green berry:

- ✓ Green colour (chlorophylle)
- ✓ Firm texture
- ✓ High concentration of acids (20 g/Kg)
- ✓ Fixates atmospheric CO_2
- ✓ Produces sugars, starch, organic acids, phenolics, etc., just like the vine leaves do

Viticulture – Grape chemistry

Grape berry: evolution of maturation

- **Second period: *"véraison"***
  (the onset of grapes ripening – the change of color of the grape berries)

During the maturation/ripening the berries:

- ✓ Become softer
- ✓ Develop colour very fast (1 day for each berry - 15 days for all the vine)
- ✓ Their concentration in sugar increases

Viticulture – Grape chemistry

Grape berry: evolution of maturation

- **Maturation/ripening:**
  - ✓ The period until full maturity lasts 40-50 days
  - ✓ Acidity decreases and sugars accumulate
  - ✓ The berries receive nothing more from the leaves
  - ✓ Fructose increases & glucose decreases (ratio reaches 0.95)
  - ✓ The grapes are ripe when their seeds acquire the ability to germinate

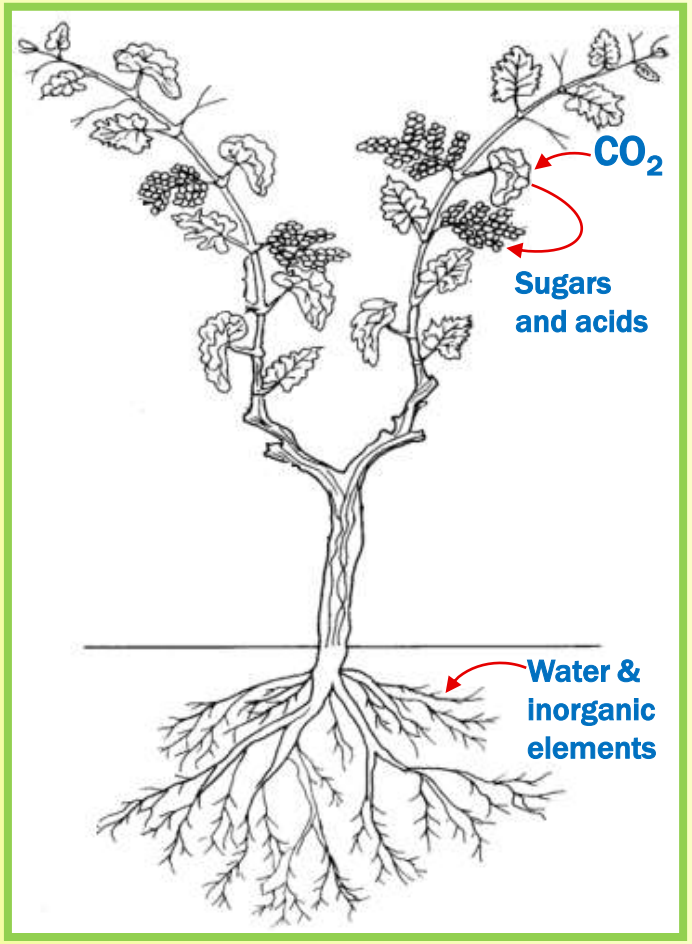
Viticulture – Grape chemistry

Grape berry: evolution of maturation

Technological maturation

It is defined as the suitable time to **harvest** the grapes and is determined by several factors:

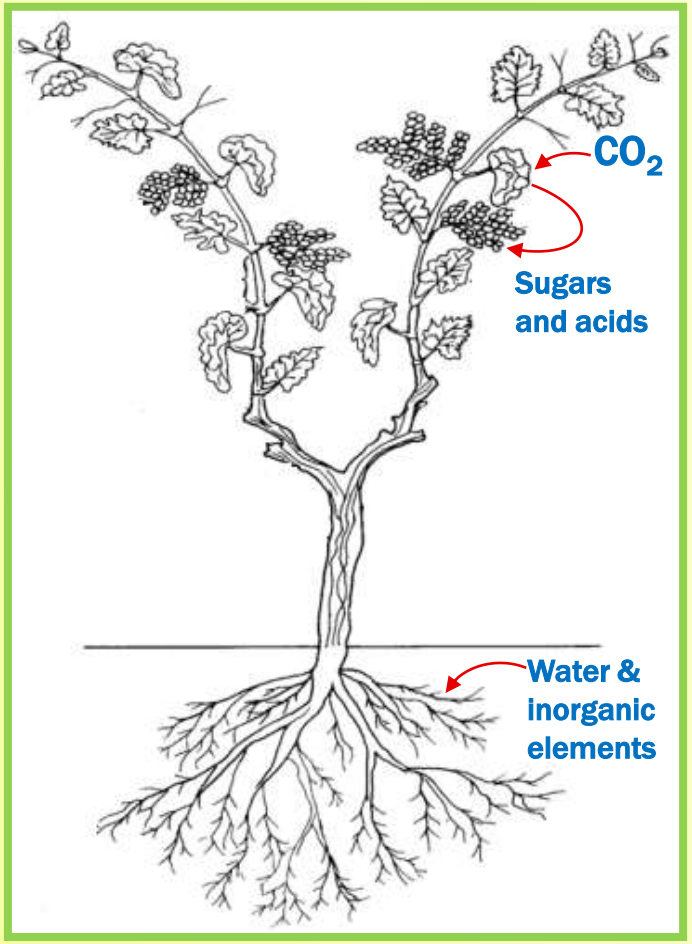
- In **warm climates**: harvest takes place **early**, before the sugars reach their maximum concentration so that a desirable acidity is retained
- In **cold climates**: harvest takes place **later**, so that a slight concentration of sugars occurs due to evaporation, and the organic acid content is reduced due to partial **malolactic fermentation** (conversion of malic acid to lactic acid)



Grape berry: evolution of maturation

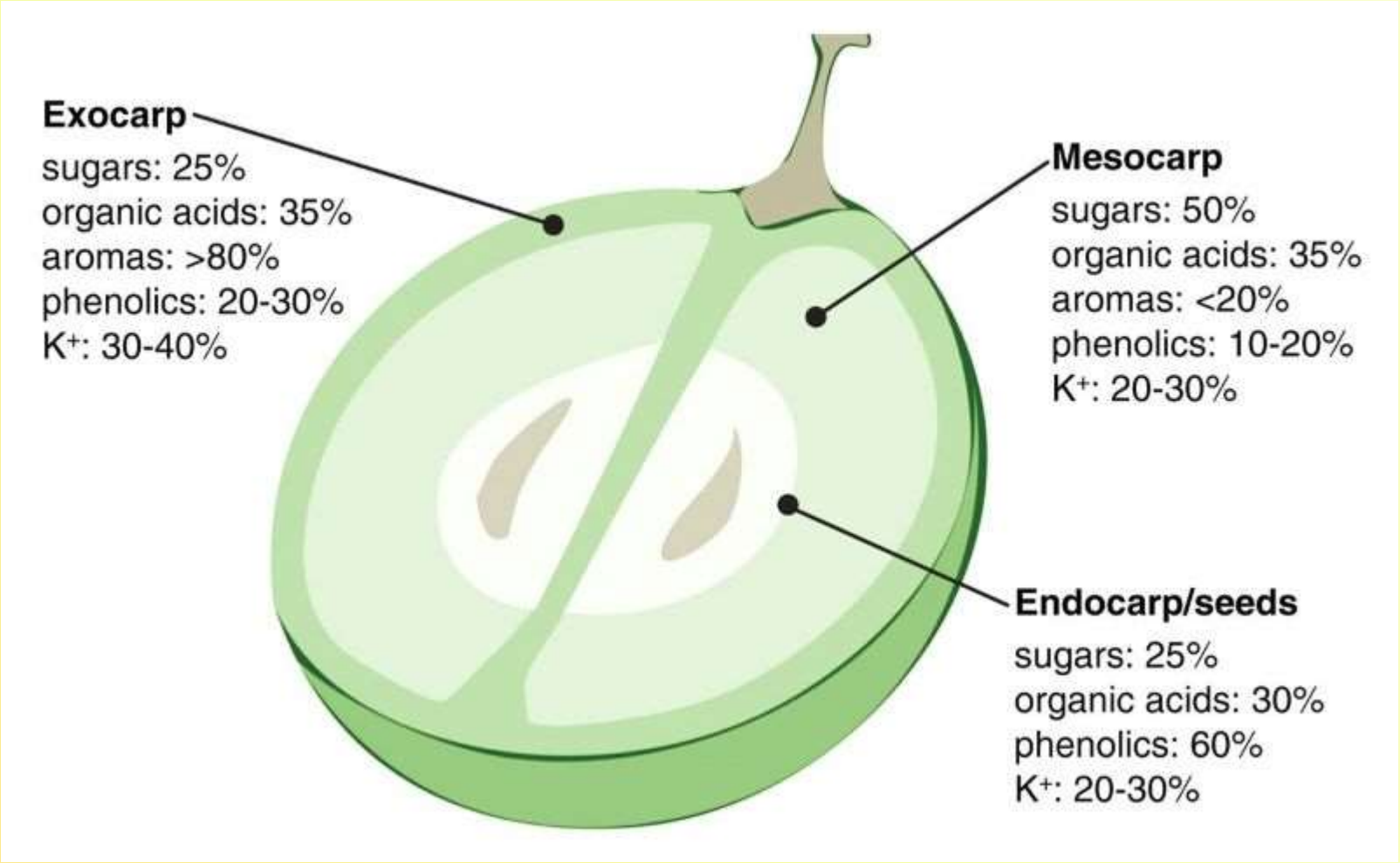
Technological maturation

- There is also a maturation level determined by the content of phenolic and aroma components
- It reaches its optimum at different times due to different biological mechanisms



Viticulture – Grape chemistry

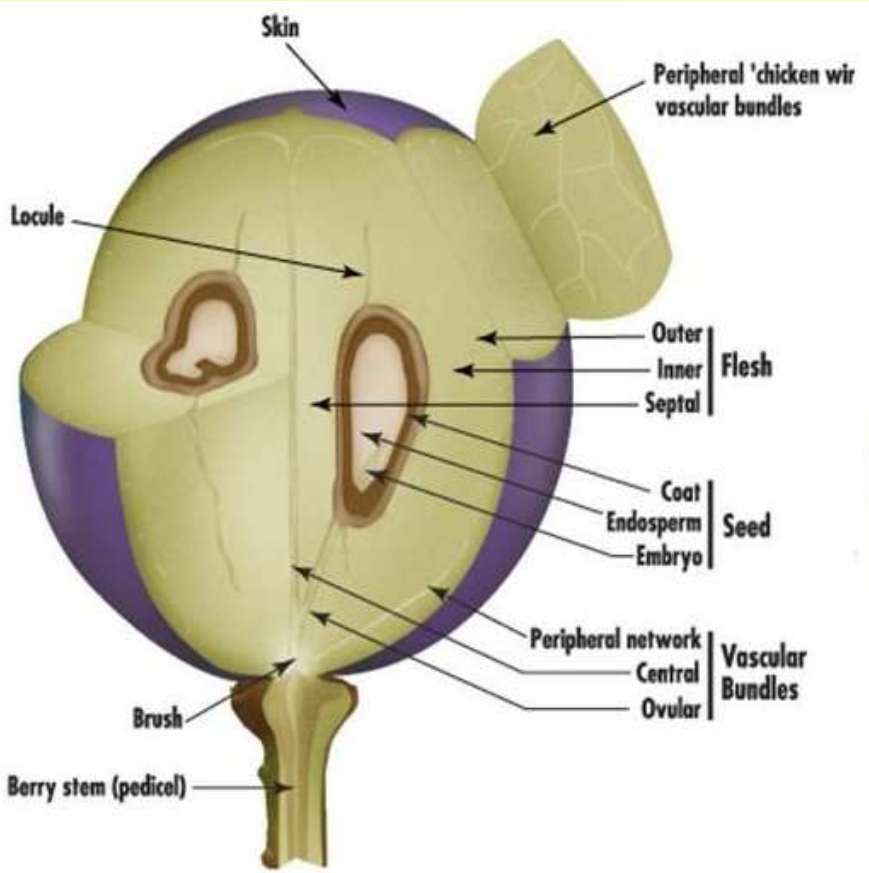
Grape berry



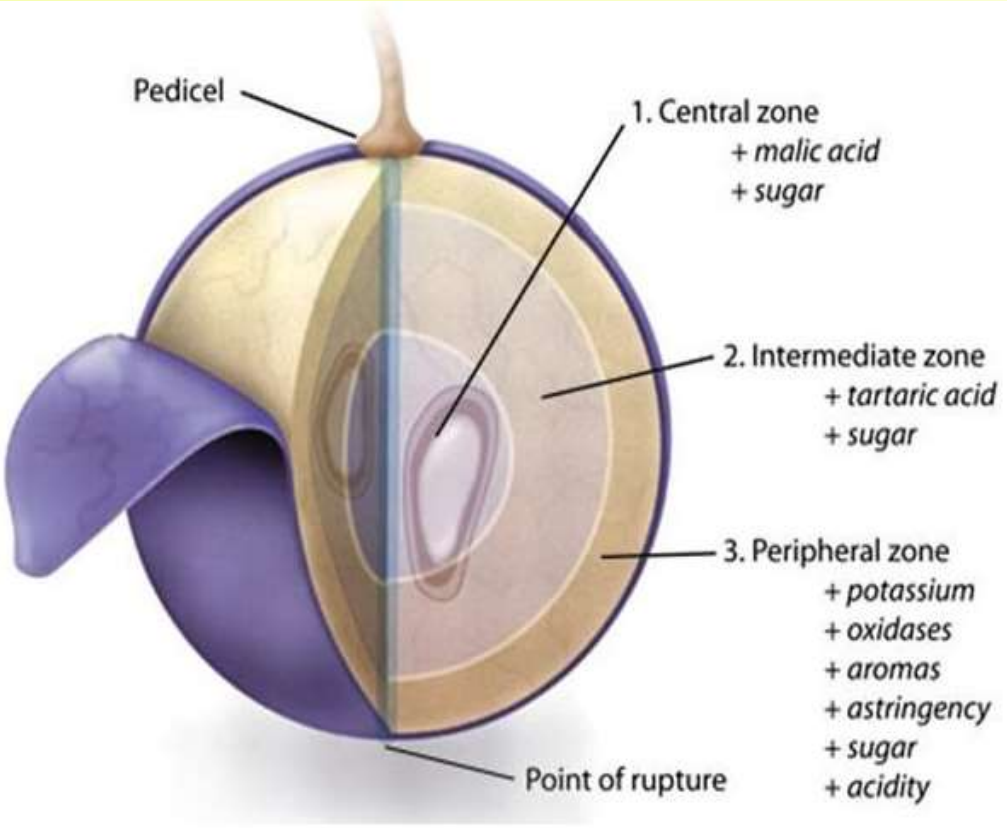
https://www.ajevonline.org/content/62/3/270.figures-only

Viticulture – Grape chemistry

Grape berry



Grape Berry Diagram[1]

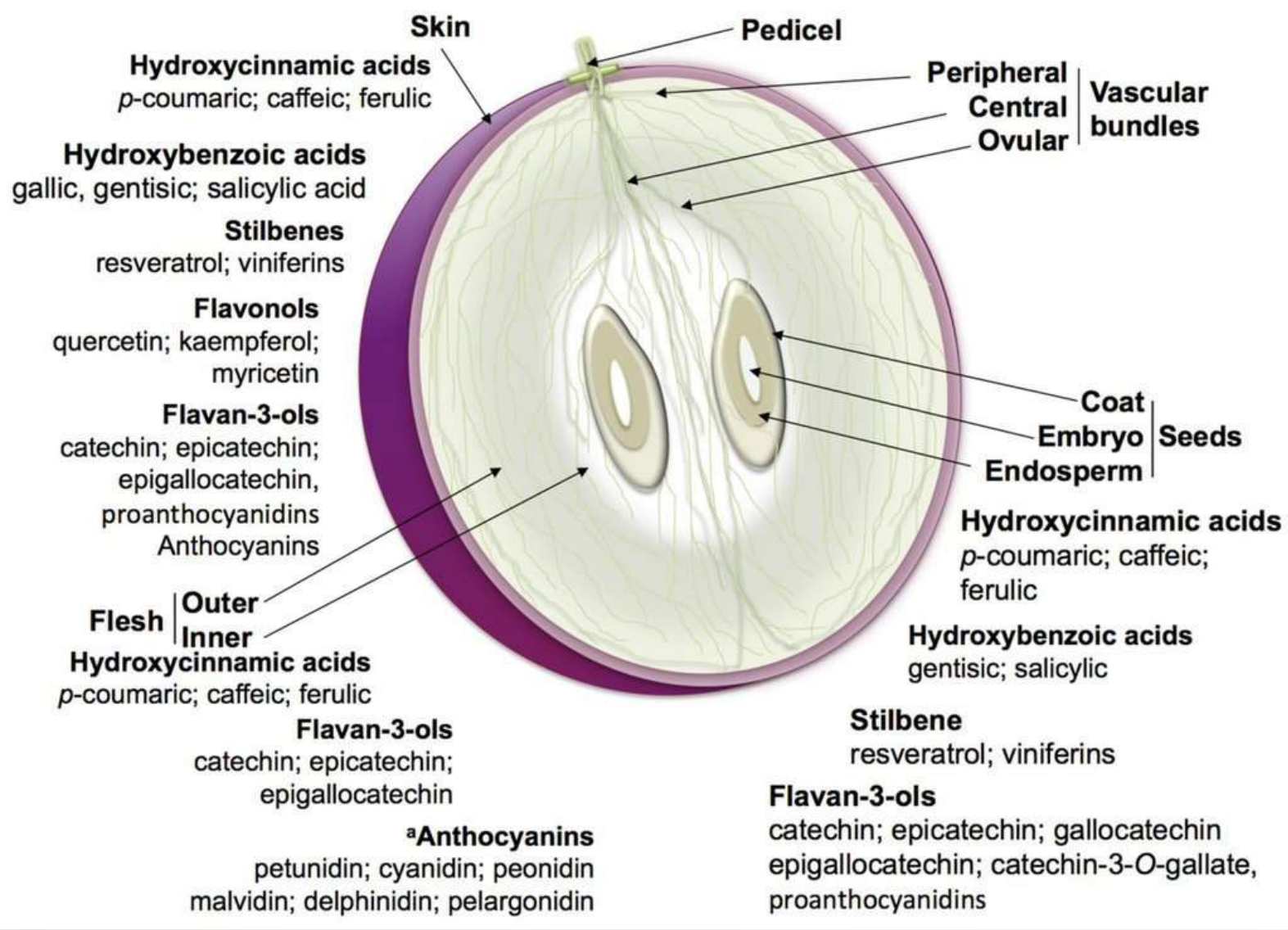


Grape Berry Flavor Zones[2]

https://gr.pinterest.com/pin/350858627197962403/

Viticulture – Grape chemistry

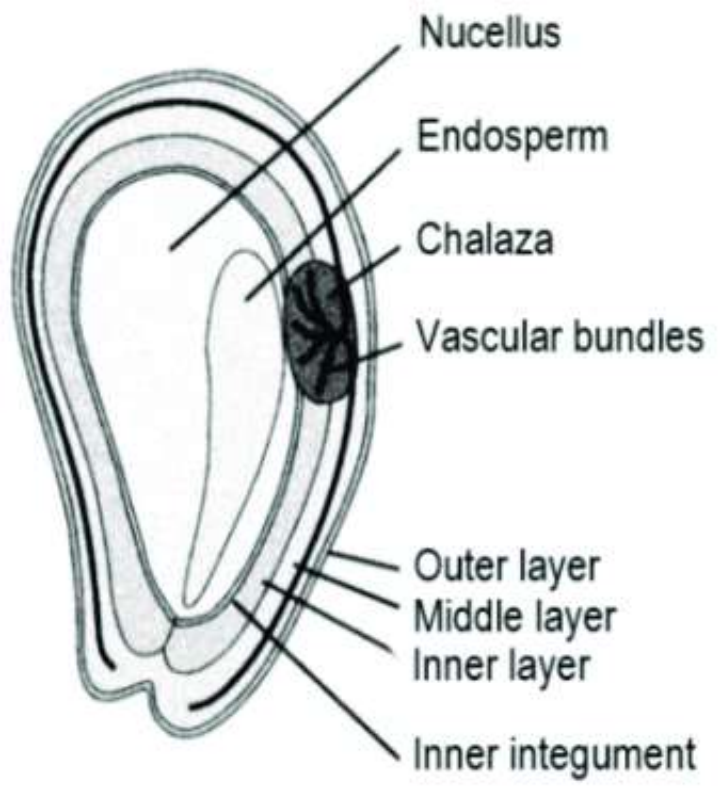
Grape berry



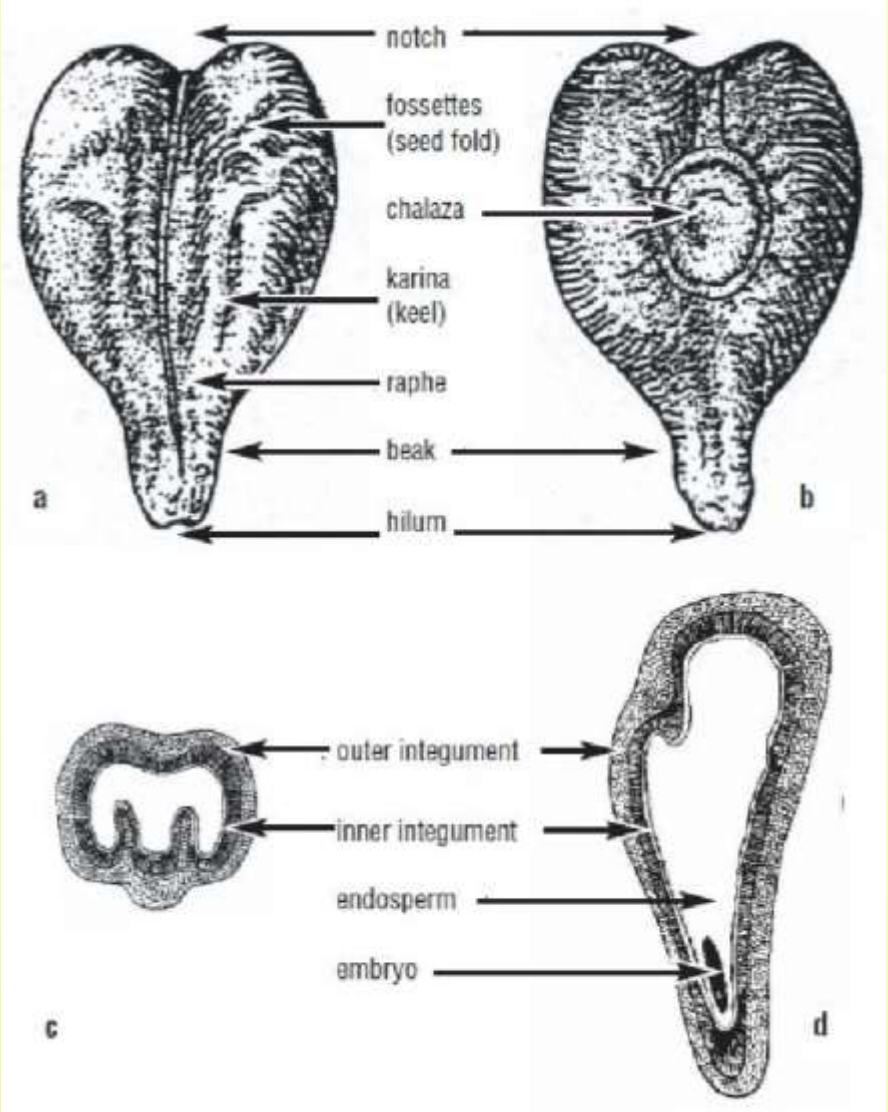
Distribution of phenolic compounds: https://twitter.com/DrJimsWine/status/722136362896392192/photo/1

Viticulture – Grape chemistry

Grape berry - seeds



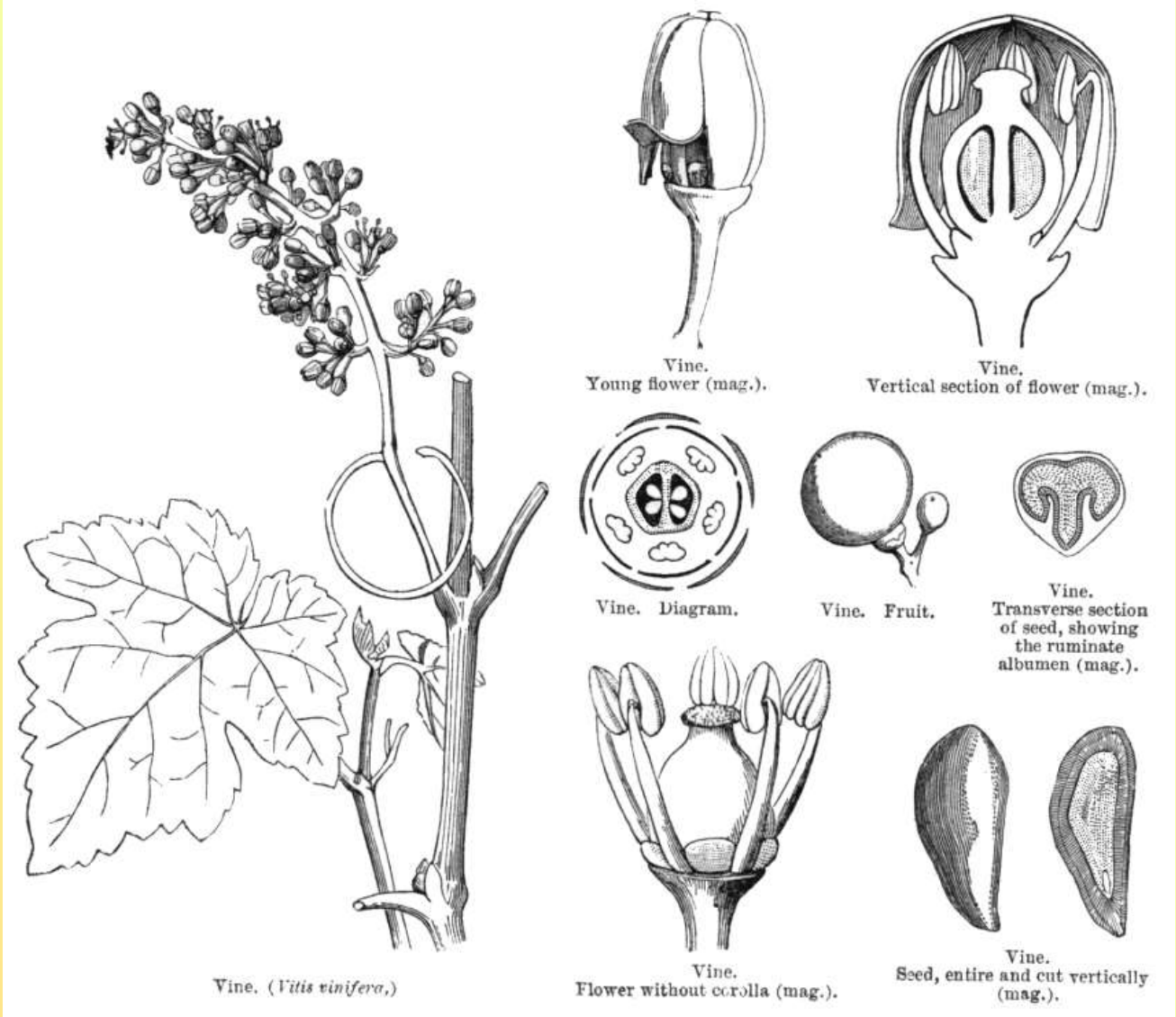
https://www.researchgate.net/publication/350901322_The_Potential_of_Grape_Pomace_Varieties_as_a_Dietary_Source_of_Pectic_Substances/figures



https://www.researchgate.net/publication/50369895_The_influence_of_different_winemaking_techniques_on_the_extraction_of_grape_tannins/figures?lo=1

Viticulture – Grape chemistry

Grape berry - seeds



Seed composition:

- Water 24-45%
- Carbohydrates 35%
- Lipids 13-20%
- Tannins 4-6%
- Nitrogen compounds 4-6.5%
- Inorganic elements 2-4%
- Fatty acids 1%

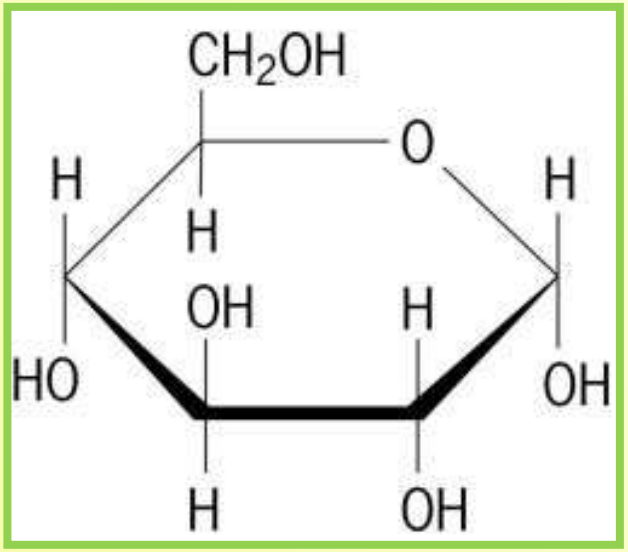
https://www.delta-intkey.com/angio/images/vitid349.gif

Viticulture – Grape chemistry

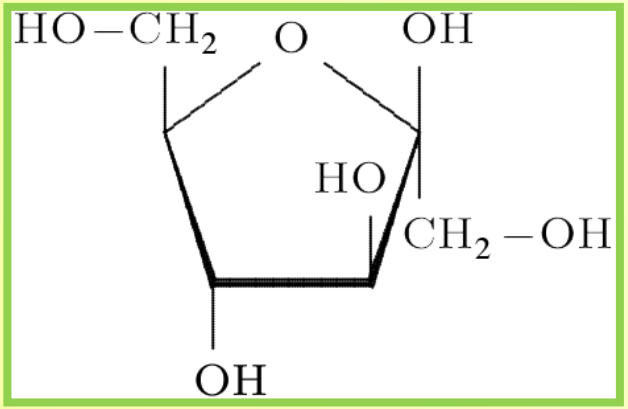
Grape berry composition - carbohydrates

Hexoses:

- ***D-glucose*** & ***D-fructose***
- During maturation the fructose content increases over glucose due to the activity of the enzyme ***epimerase***
- **total: >150-250 g/L** (may increase due to berry dehydration or by *"noble rot" from Botrytis cinerea*)
- **Residual sugar (~1 g/L)** during wine making is usually due to the presence of fructose
- The sugar content depends on many factors such as: maturation level, soil and climate conditions, cultivation practices, canopy management, type of rootstock, fertilization, irrigation, etc.



D-glucose



D-fructose

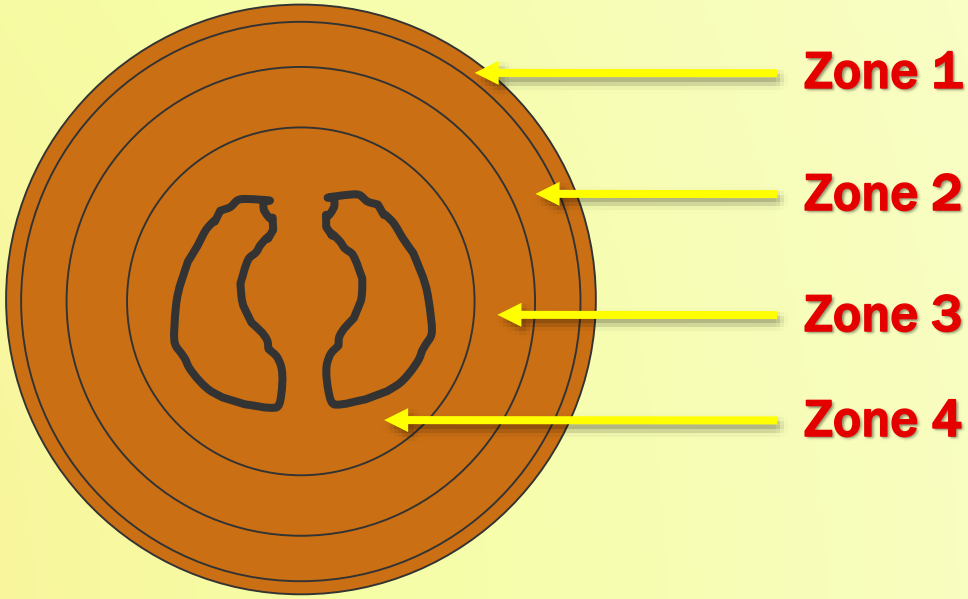
Viticulture – Grape chemistry

Grape berry composition - carbohydrates

Hexoses:

- The distribution of sugars in the berries is uneven (Figure) so that white vinification the first grape juice that is received [terms: “first pressing”, “*free run*”, “*primera yema*”, “*vin de goutte (wine)*”], has a different composition, depending on the way of pressing, from the must obtained by the next pressings
- The first run is richer in sugars and the sugar content decreases with the successive pressings

Zone	Sugars g/L	Tartaric acid g/L	Malic acid g/L	K g/L	Ca g/L
1	125	5,5	10,2	7,5	5,5
2	175	4,4	1,2	2,5	1,5
3	127	6,1	2,5	2,0	2,0
4	225	8,0	8,0	4,0	3,5

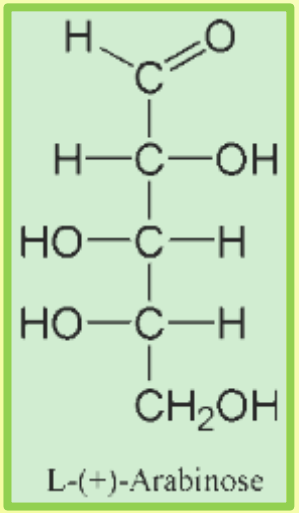


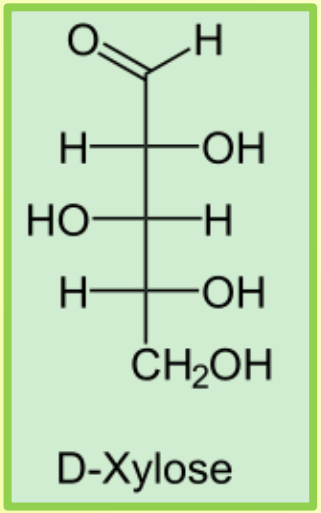
Viticulture – Grape chemistry

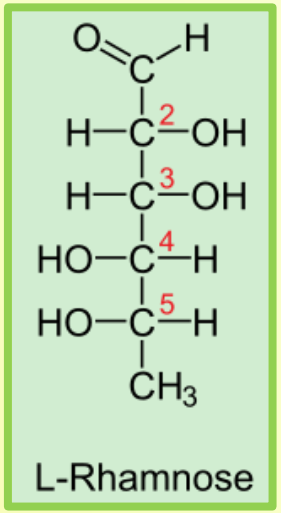
Grape berry composition - carbohydrates

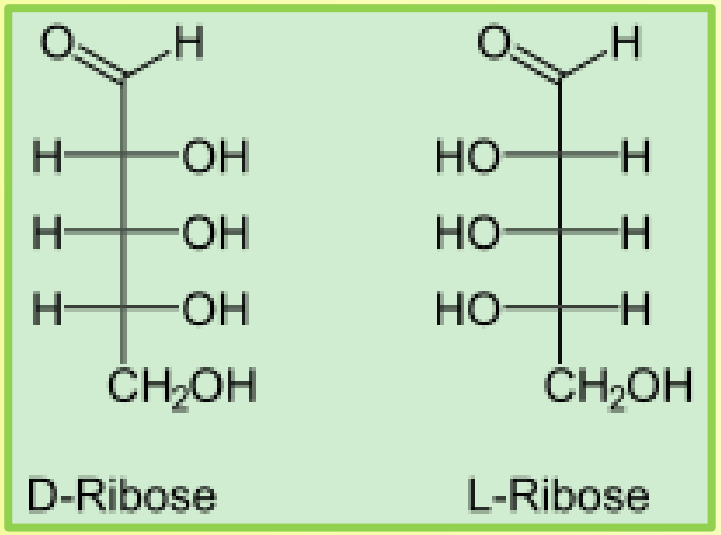
Other sugars:

- Pentoses*: L-arabinose, D-xylose, D- & L-ribose*
- *L-rhamnose (in Muscat varieties)*)
- Total: **0,3-2 g/L**
- Low sweetness
- Are not fermented by yeasts and are mainly found in red wines
- 4-C sugars have not been found in grapes







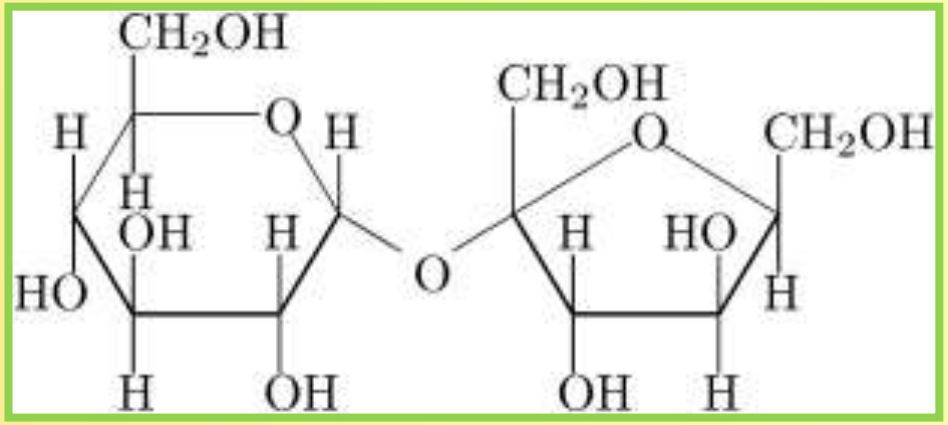


Viticulture – Grape chemistry

Grape berry composition - carbohydrates

Disaccharides:

- **Sucrose** (it is enzymatically produced by binding of glucose+fructose by the enzyme *synthetase*)
- Total: **2-5 g/L**
- It is hydrolyzed in its monomers by the action the enzyme *invertase* (which is found in high amounts in the grape berry)
- It can be fermented by yeasts after hydrolysis to its monomers



Viticulture – Grape chemistry

Grape berry composition - carbohydrates

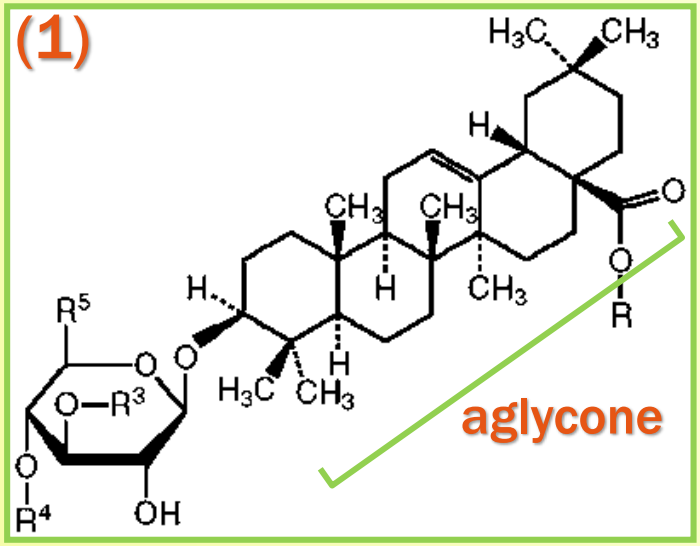
Glucosides

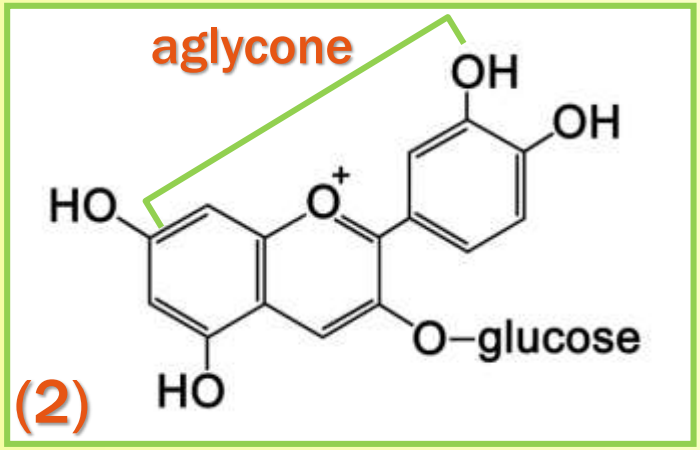
Compounds consisting of carbohydrates bound to other non-carbohydrate compounds (*aglycones*)

For example:

- ✓ ***Oleanolic acid glucoside.*** An pentacyclic triterpenoid that contributes to the alcohol resistance of yeasts during fermentation (1)
- ✓ ***Terpenol glucosides*** that are precursors of aroma compounds (linalool, nerol, geraniol, citronellol, etc.) after hydrolysis of the aglycone fraction
- ✓ ***Anthocyans*** that are the grape pigments (2)

(1)



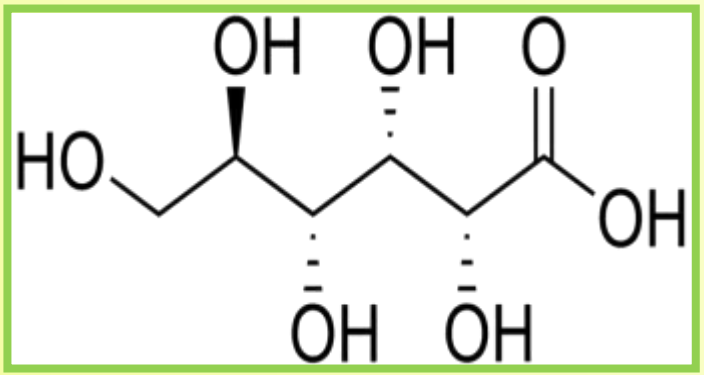


Viticulture – Grape chemistry

Grape berry composition - carbohydrates

Sugar oxidation products

- ***Gluconic acid*** that derives from the oxidation of the glucose carbonyl group
- It is usually found due to contamination by *Botrytis cinerea*



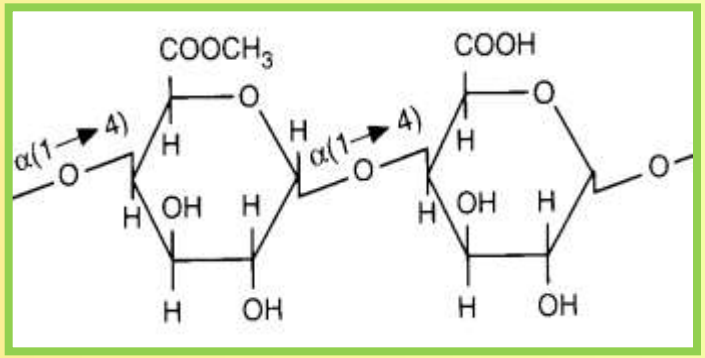
Gluconic acid

Grape berry composition - carbohydrates

Pectin substances

Polysaccharides, grape skin and cell wall components

- ✓ ***Polygalacturonic acid:*** a partially esterified polymer (with methanol; 70-80% methyl ester groups) of α-D-galacturonic acid (α-1,4 glucosidic bonds)



Pectin

Grape berry composition - carbohydrates

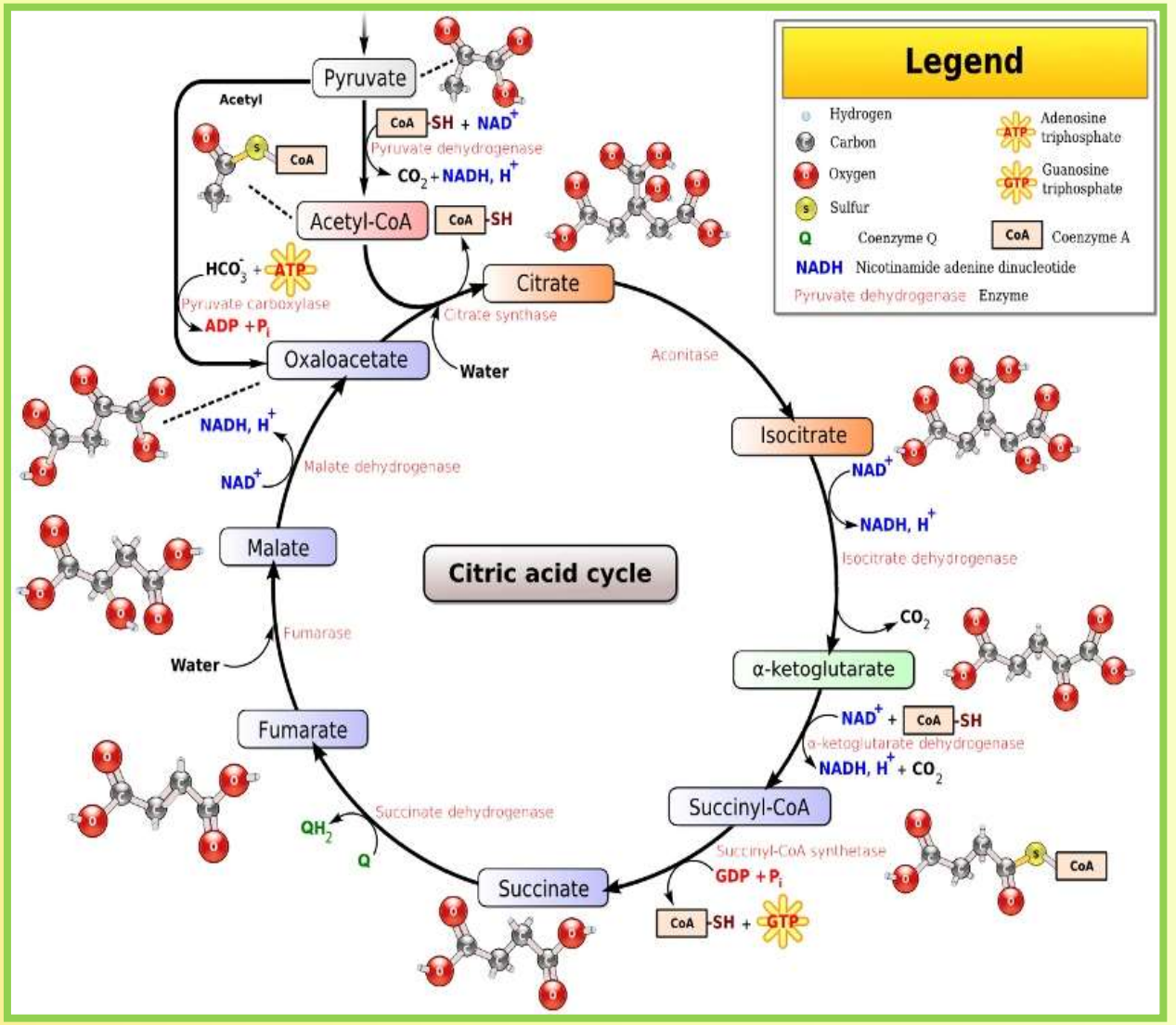
Changes in polysaccharide composition during maturation

- ✓ ***Pectins***
- ✓ ***Cellulose***
- ✓ ***Hemicelluloses***
  - They are contained at a very low content in grape juice
  - They are mainly components of the cell walls
  - During maturation, intense hydrolysis of cell walls and an increase in the concentration of soluble polysaccharides are observed.

Viticulture – Grape chemistry

Grape berry composition – organic acids

- The main organic acids of the berries are tartaric, malic and to a lesser extent citric.
- There are plenty of other acids at lower amounts (Krebs cycle products)

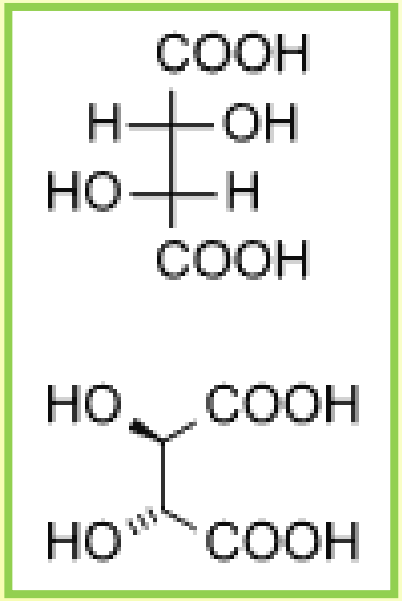


Viticulture – Grape chemistry

Grape berry composition – organic acids

L-(+)-tartaric acid (wine acid):

- ✓Product of the secondary metabolism of sugars
- ✓It is composed in the leaves and berries only in the vine plant and no other species in Europe
- ✓Concentration in grape juice: northern countries 6 g/L – warmer countries 2-3 g/L
- ✓It is a relatively strong acid (juice pH 3.0-3.2)
- ✓Winemaking sludge, which is rich in tartrate, is used as raw material for the industrial production of tartaric acid with applications in the production of foods and beverages and in leather processing



L-(+)-tartaric acid

HOOC-CH(OH)-CH(OH)-COOH

Viticulture – Grape chemistry

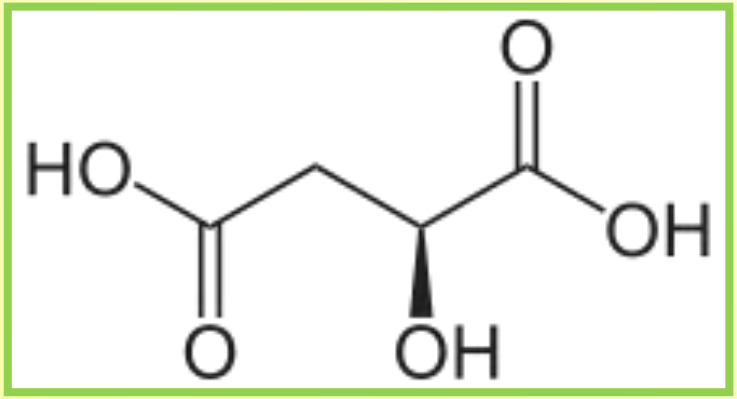
Grape berry composition – organic acids

- ***L-Malic acid (apple acid):***

✓ Product of sugar metabolism and an important intermediate of the Krebs cycle

✓ A common acid in apples

✓ Concentration in the grape berries: before maturation 25 g/L - two weeks after véraison ~12-13 g/L (due to dilution and catabolism) - at maturity 4.0- 6.5 g/L (northern countries) or 1-2 g/L (warm countries)



L-malic acid

$HOOC\text{-}CH_2\text{-}CH(OH)\text{-}COOH$

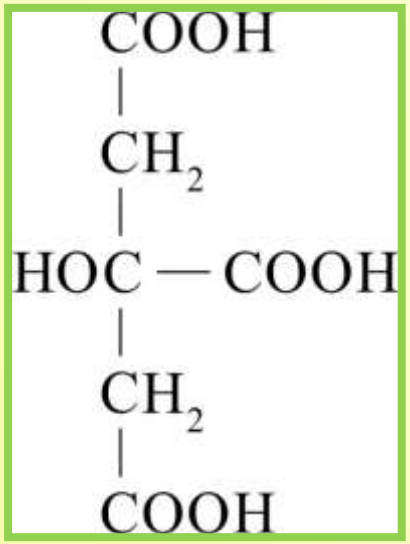
✓ It contributes to the acidity of the grape juice but is metabolized much more easily than tartrate, which ultimately defines the acidity of the juice

Viticulture – Grape chemistry

Grape berry composition – organic acids

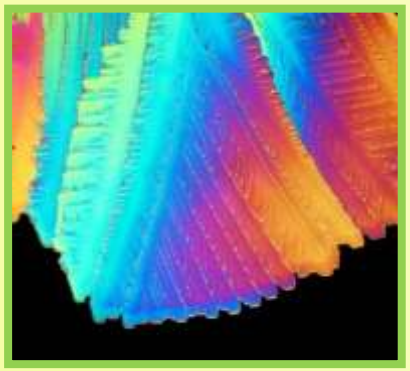
Citric acid:

- ✓ Product of sugar metabolism and an important intermediate of the Krebs cycle
- ✓ A common acid in citrus fruit
- ✓ Concentration in grape must: 0.5-1 g/L
- ✓ Contributes to the acidity of grape juice along with malic and tartaric acids
- ✓ Its salts are used in the food & pharmaceutical industries, and in photography



Citric acid

$HOOC\text{-}CH_2\text{-}CH(OH)(COOH)\text{-}CH_2\text{-}COOH$

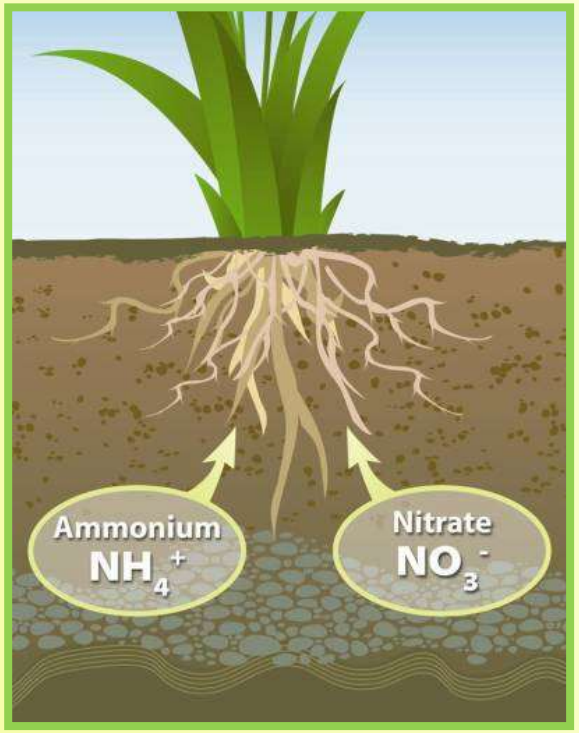


Viticulture – Grape chemistry

Grape berry composition – nitrogen compounds

Nitrogen (N):

- **80%** content in the atmosphere as N_2
- Inert gas with a variety of applications
- It is not assimilated in its molecular form by plants
- It is utilized by plants in **ionic** or **organic** forms
- **In the grape juice**: it is present as both inorganic and organic compounds - its content (total N) changes from year to year due to changes in maturity levels and is affected by the cultivation practice and vine variety

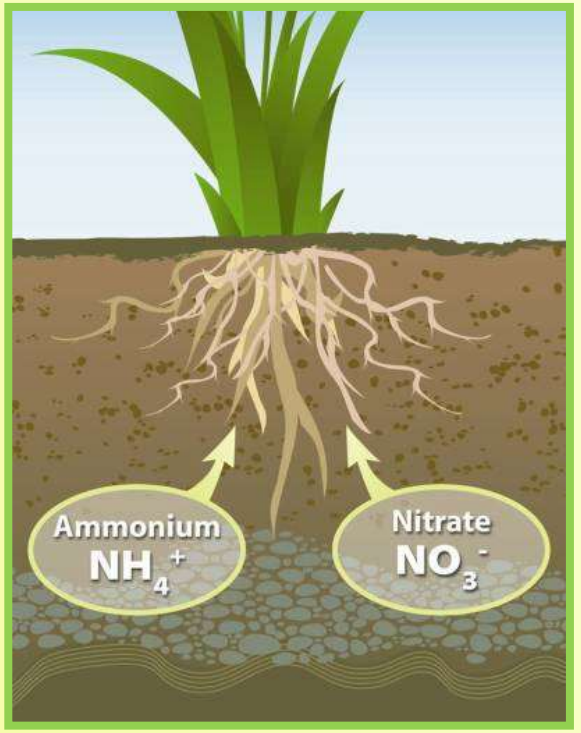


https://www.sciencelearn.org.nz/resources/960-the-nitrogen-cycle

Viticulture – Grape chemistry

Grape berry composition – nitrogen compounds

- Inorganic N in grape juice: 80% of the total N of the green berries (up to véraison) is found in the form of ammonium salts (NH_4^+) which derive from the reduction of nitrates (NO_3^-) that were absorbed by the roots
- After véraison, ammonia N is rapidly reduced due to its conversion to organic compounds (amino acids, peptides, polypeptides, proteins)
- At full maturity only 10% of total N is inorganic

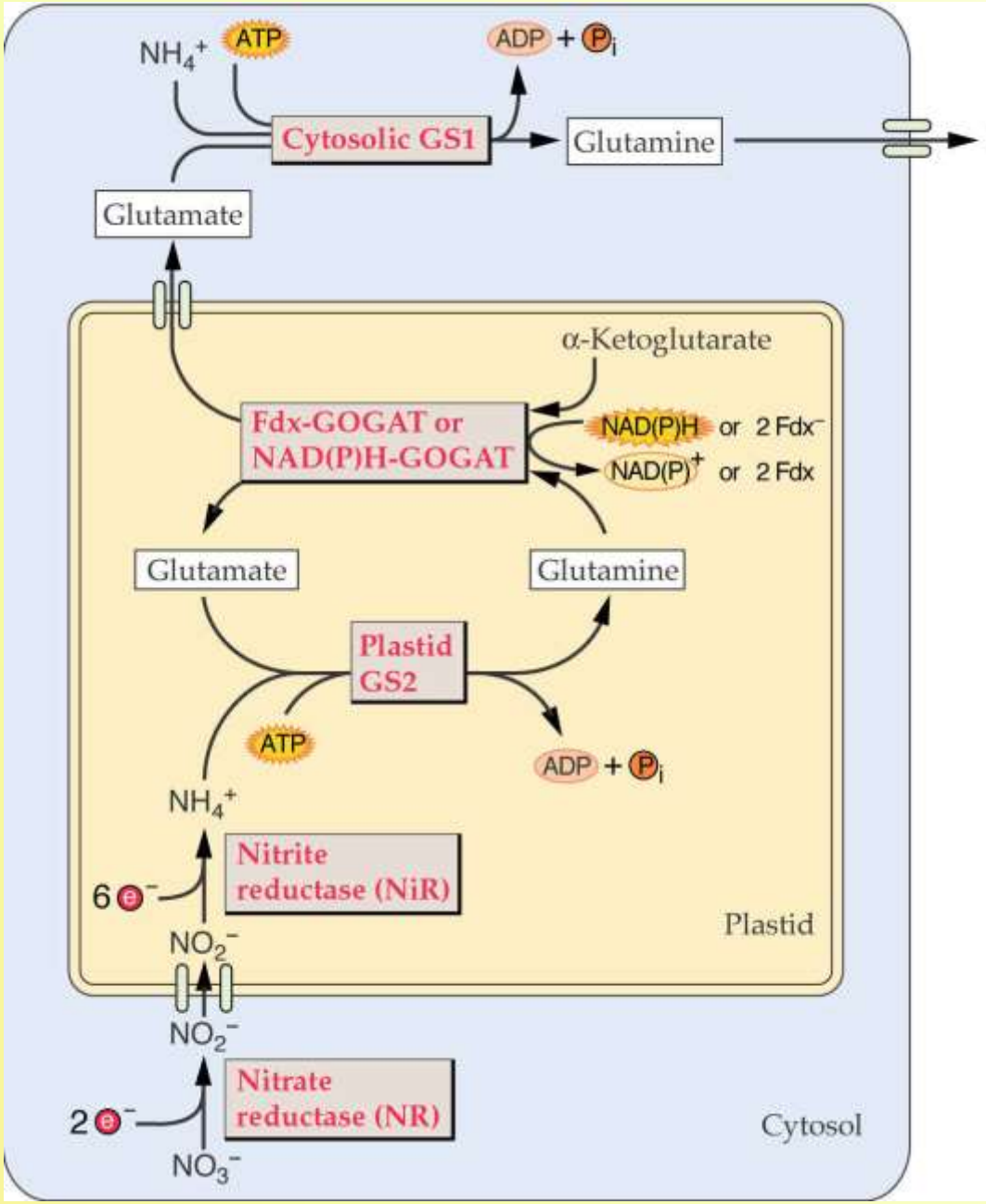


https://www.sciencelearn.org.nz/resources/960-the-nitrogen-cycle

Viticulture – Grape chemistry

Grape berry composition – nitrogen compounds

- **Ammonium ion (NH_4^+)** is the most assimilable form of nitrogen
- However, **nitrates (NO_3^-)** are the the basic source of nitrogen (**fertiliser**) that will be reduced to NH_4^+ by the plant in order to be assimilated



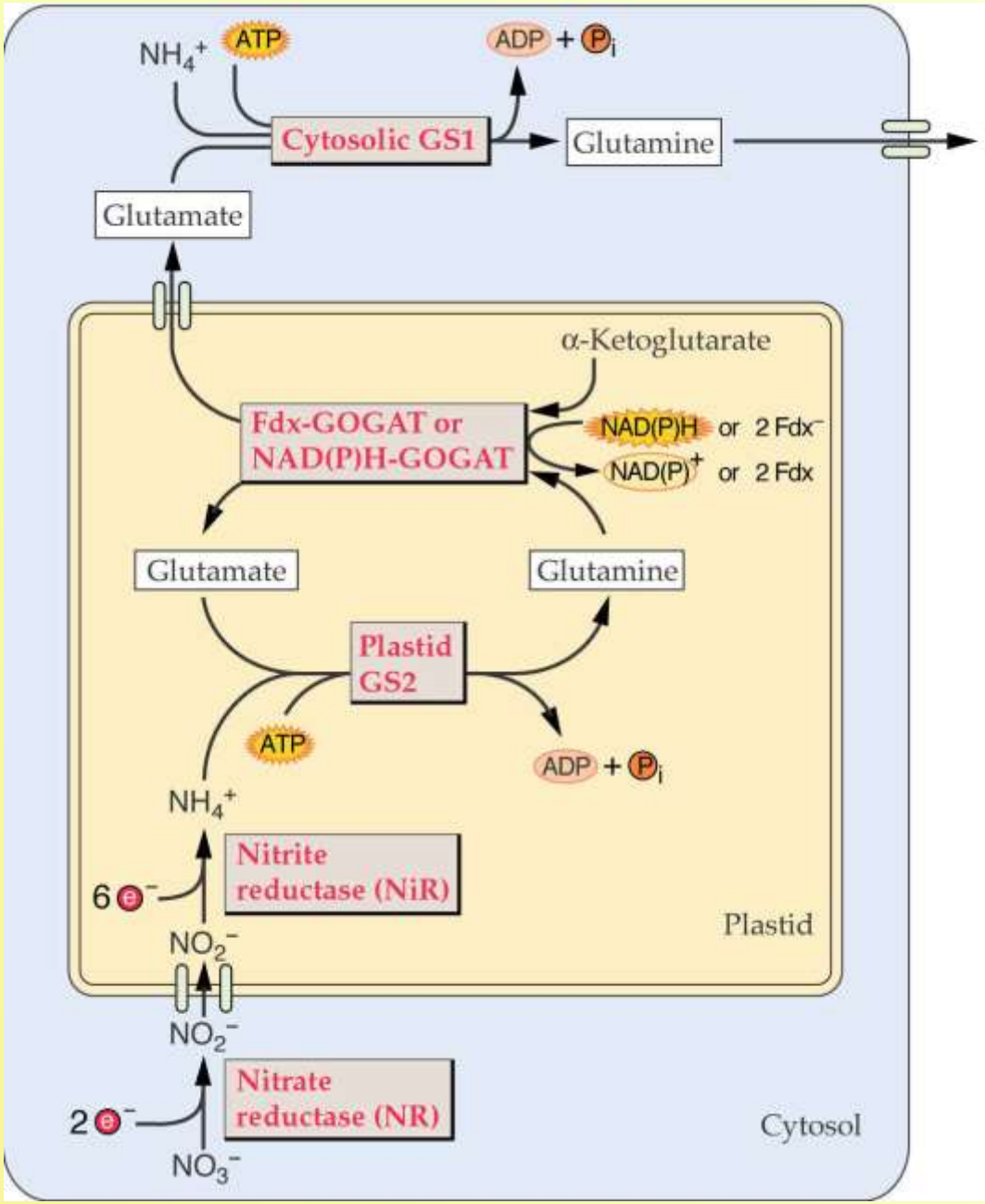
http://www.uky.edu/~dhild/biochem/24/lect24.html

Viticulture – Grape chemistry

Grape berry composition – nitrogen compounds

- The concentration of NH_4^+ in grape juice (must) affects the start and rate of the alcoholic fermentation during the wine making process

- When the concentration of NH_4^+ is lower than **50 mg/L,** then diammonium phosphate $(NH_4)_2HPO_4$ or ammonium sulfate $(NH_4)_2SO_4$ are added in the grape must at **~10 g/100 L**



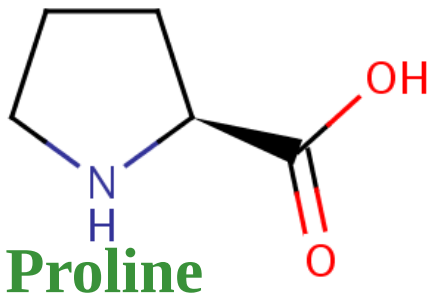
http://www.uky.edu/~dhild/biochem/24/lect24.html

Viticulture – Grape chemistry

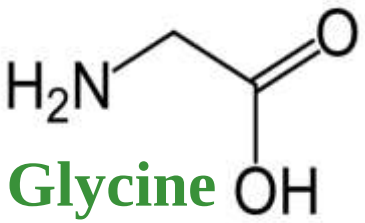
Grape berry composition – nitrogen compounds

Organic N – Amino acids

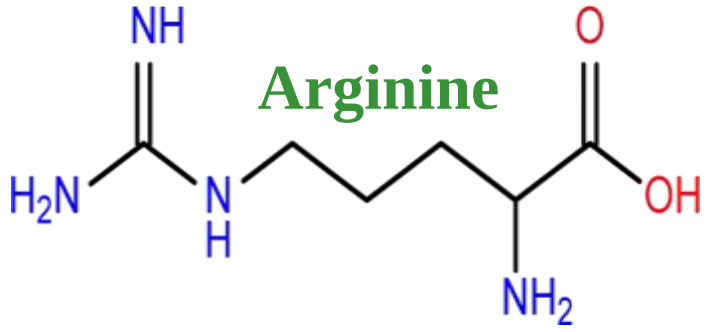
- The most common of organic N in the mature grape (**1 - 4 g/L; 30-40% of total N**)
- A spectacular increase in **proline** is observed 2 weeks before maturation: maturation indicator
- Other amino acids at high levels: **arginine, glycine, alanine, serine**



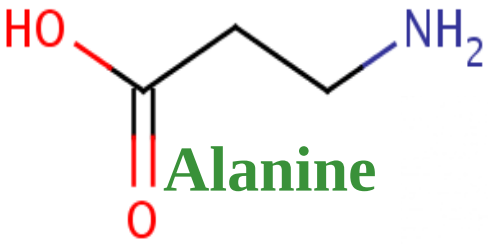
Proline



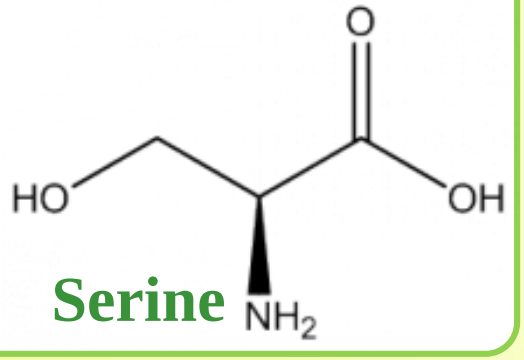
Glycine



Arginine



Alanine



Serine

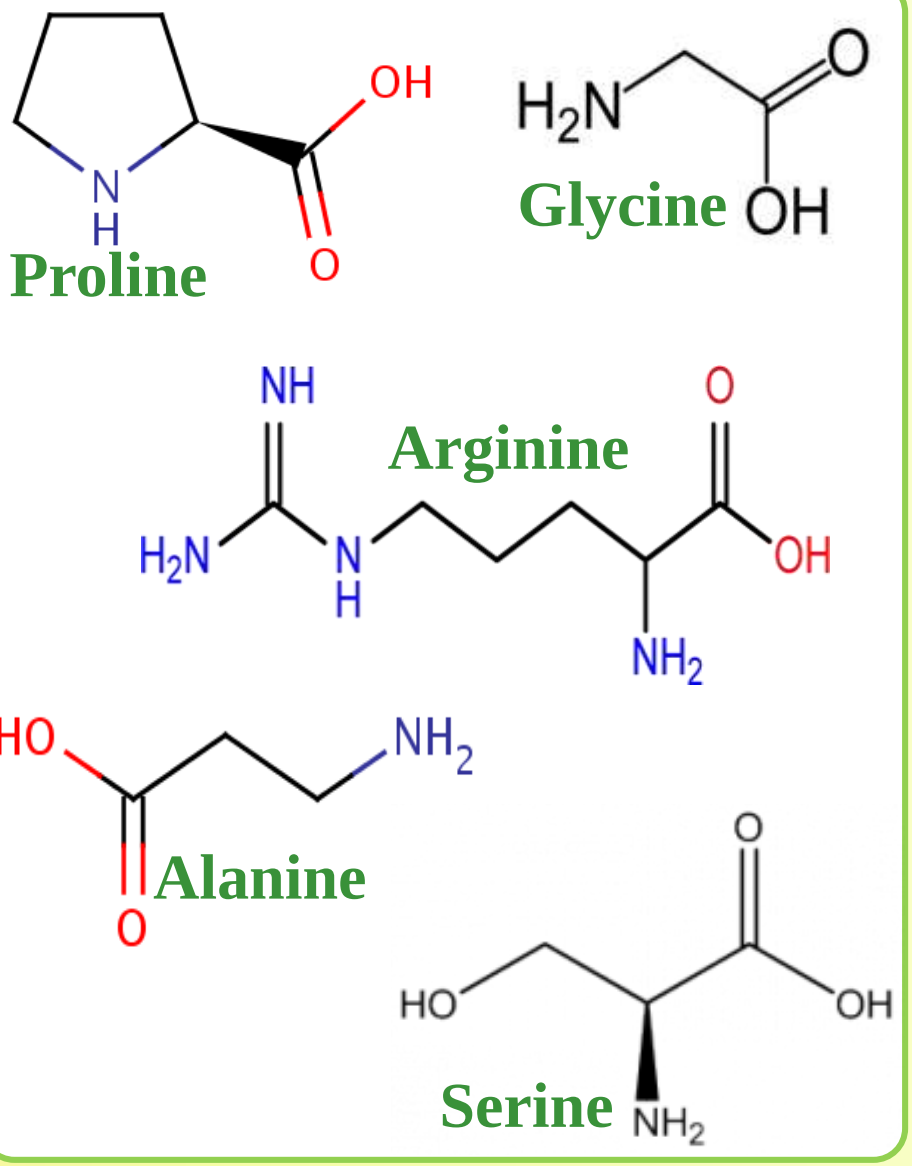
Viticulture – Grape chemistry

Grape berry composition – nitrogen compounds

Organic N – Amino acids

- Amino acids are utilized as nutrients for yeasts during the alcoholic fermentation and are metabolized into products that affect the sensory properties of wine (**aldehydes, katons, esters, alcohols**)

- The amino acid profile in grape must has been studied in correlation with the grape variety and the geographical origin of the vine



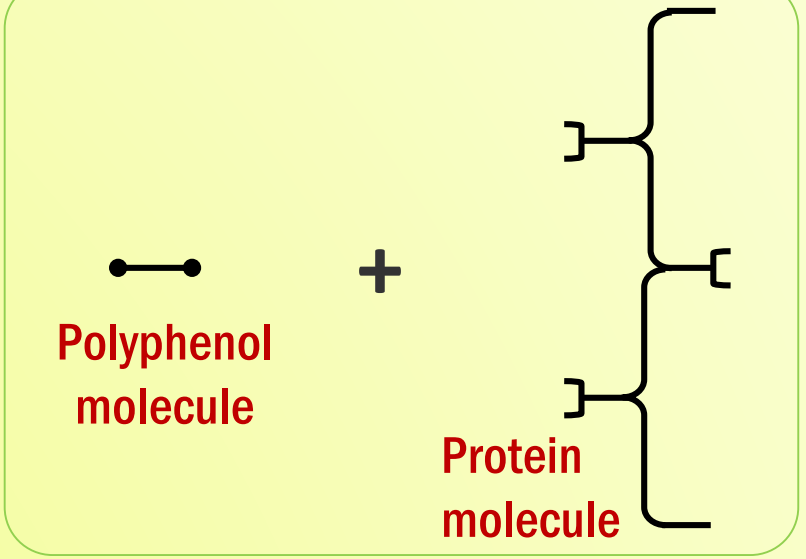
Viticulture – Grape chemistry

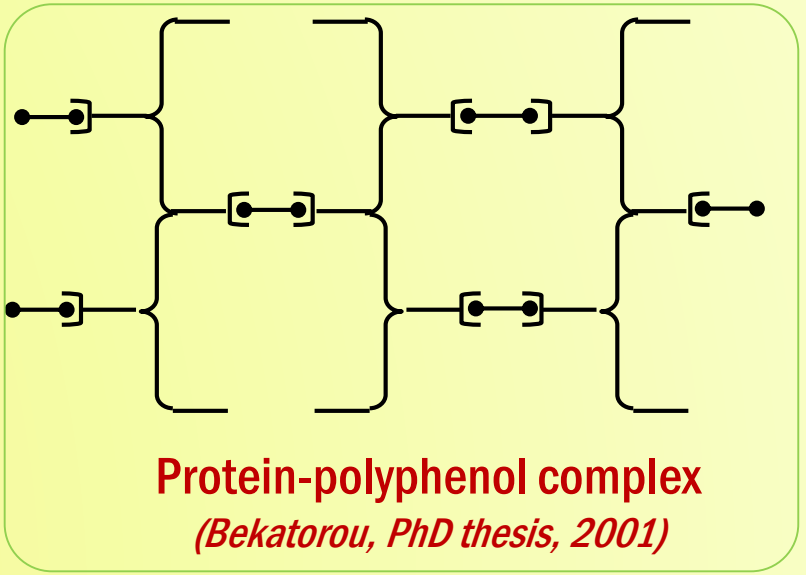
Grape berry composition – nitrogen compounds

Organic N-Oligopeptides/polypeptides

- oligopeptides: 4 amino acids
- polypeptides: MW <10.000
- proteins: MW >10.000

- Proteins in wines/grapes found at: MW range 30.000-150.000
- **Red wines**: lower levels of proteins due to precipitation by interaction with tannins (polyphenols)
- **White/rosé wines**: protein levels of ~100 mg/L that may cause "protein hazes" and require stabilization with several protein removal (clarification, racking)





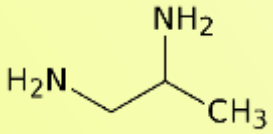
Protein-polyphenol complex

(Bekatorou, PhD thesis, 2001)

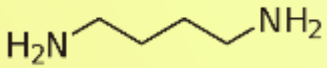
Grape berry composition – nitrogen compounds

Organic N - Polyamines (Pas)

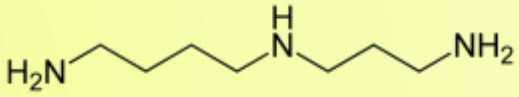
- Aliphatic amines with 2 or more amino groups
- The main polyamines of plants are diamines & triamines:
  - 1,3-Diaminopropane $H_2N\text{-}(CH_2)_3\text{-}NH_2$



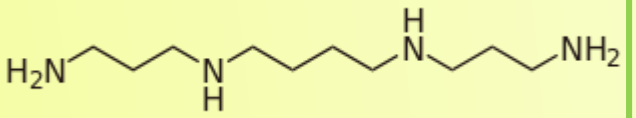
  - Putrescine $H_2N\text{-}(CH_2)_4\text{-}NH_2$



  - Spermidine $H_2N\text{-}(CH_2)_4\text{-}NH\text{-}(CH_2)_3\text{-}NH_2$



  - Spermine $H_2N\text{-}(CH_2)_3\text{-}NH\text{-}(CH_2)_4\text{-}NH\text{-}(CH_2)_3\text{-}NH_2$



Grape berry composition – nitrogen compounds

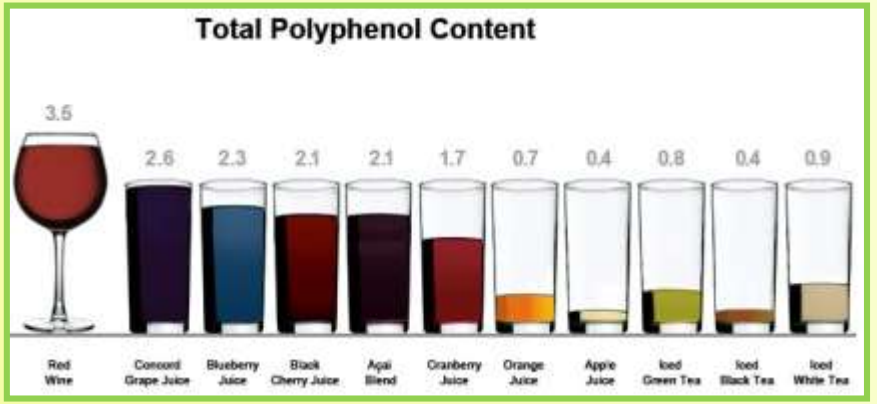
Organic N - Polyamines (Pas)

- They have positive charges (are strongly protonated at normal pH)
- They form complexes with negatively charged molecules such as nucleic acids, phospholipids, cell wall components, proteins, enzymes, etc.
- They are among the plant “growth regulators“
- Their composition in the plant is caused by various "stresses", including potassium deficiency

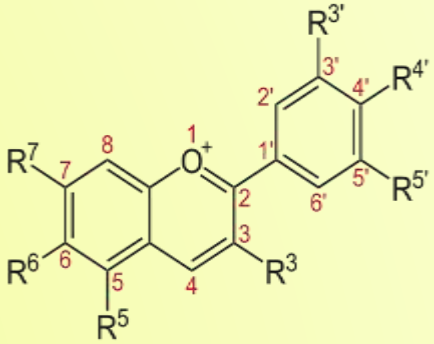
Viticulture – Grape chemistry

Grape berry composition – Phenolic compounds

- Compounds with one or more phenolic groups
- Decisive role in the quality of wine products
- Antioxidant, antiinflamatory, & antimicrobial properties; protection of the cardiovascular system
- They are contained in different parts of the grape and are extracted during vinification



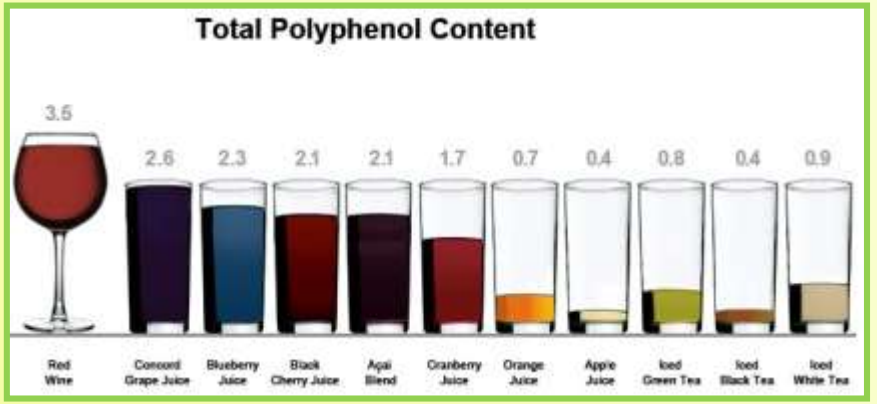
Red, purple & blue pigments (flavonoids, anthocyanins)



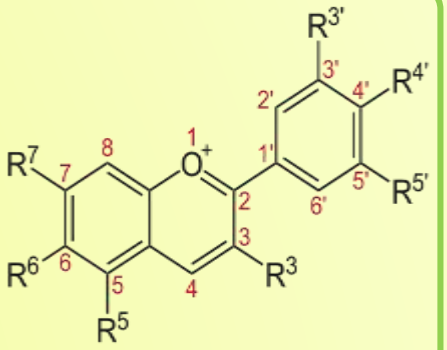
Viticulture – Grape chemistry

Grape berry composition – Phenolic compounds

1. Phenolic acids
2. Stilbenes
3. Flavonoids
4. Anthocyans
5. Catechins
6. Tannins



Red, purple & blue pigments (flavonoids, anthocyanins)

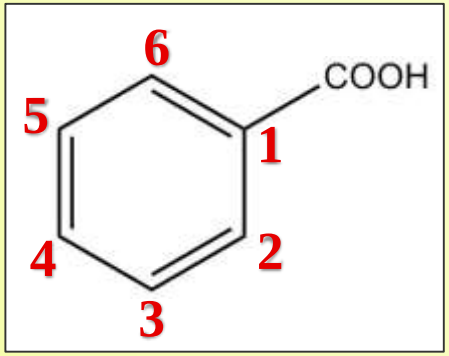


Viticulture – Grape chemistry

Grape berry composition – Phenolic compounds

Phenolic acids

- Non-flavonoid phenolic compounds - monomolecular derivatives of benzoic & cinnamic acids
- Upon maturation, 6 benzoic acid derivatives are formed:
  - ✓ Salicylic acid
  - ✓ p-Hydroxybenzoic acid
  - ✓ Gallic acid
  - ✓ Protocatechuic acid
  - ✓ Vanillic acid
  - ✓ Syringic acid



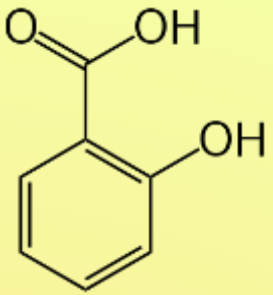
Benzoic acid

Positions of –OH or –OCH_3 groups in phenolic acids derived from benzoic acid

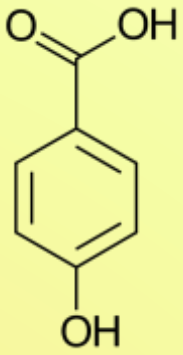
benzoic acid derivatives	-OH	-OCH_3
Salicylic	2	-
p-Hydroxybenzoic	4	-
Gallic	3,4,5	-
Protocatechuic	3,4	-
Vanillic	4	3
Syringic	4	3,5

Viticulture – Grape chemistry

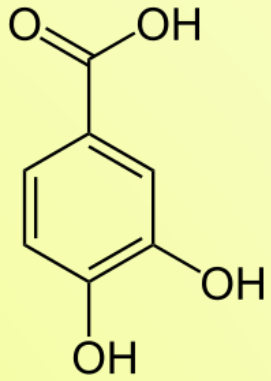
Grape berry composition – Phenolic compounds



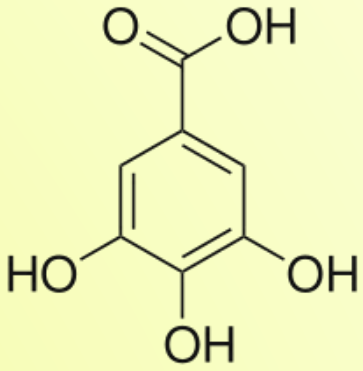
Salicylic acid



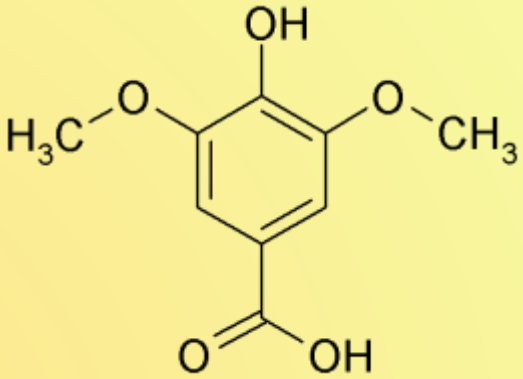
p-Hydroxybenzoic acid



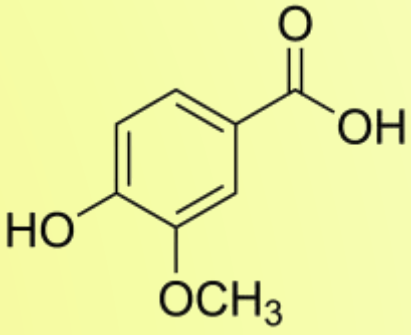
Protocatechuic acid



Gallic acid



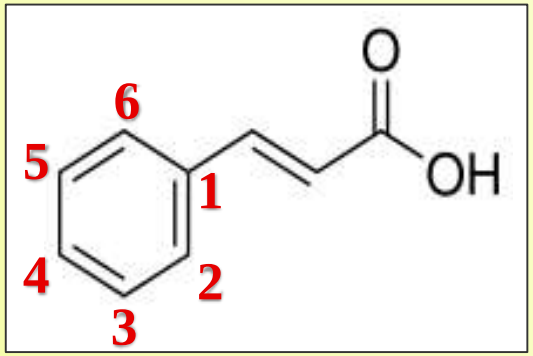
Syringic acid



Vanillic acid

Viticulture – Grape chemistry

Grape berry composition – Phenolic compounds



Cinnamic acid

Phenolic acids

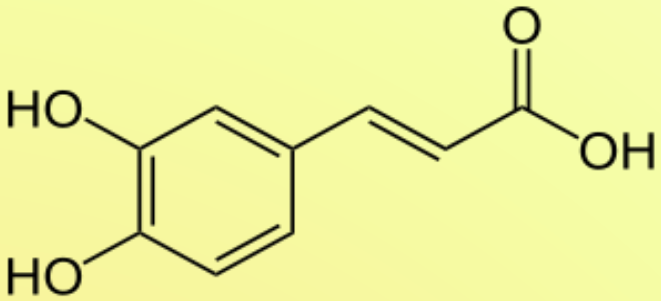
- Upon maturation, 4 cinnamic acid derivatives are formed:
  - p-cumaric acid
  - Caffeic acid
  - Chlorogenic acid
  - Ferulic acid

Positions of –OH or –OCH_3 groups in phenolic acids derived from caffeic acid

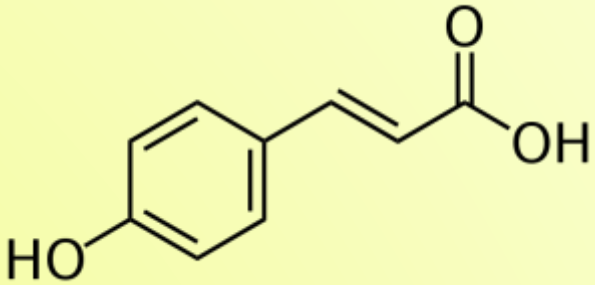
Κινναμωμικό	-OH	-OCH_3
Ferulic	4	3
p-Cumaric	4	-
Caffeic	3,4	-
Chlorogenic	3,4	-

Viticulture – Grape chemistry

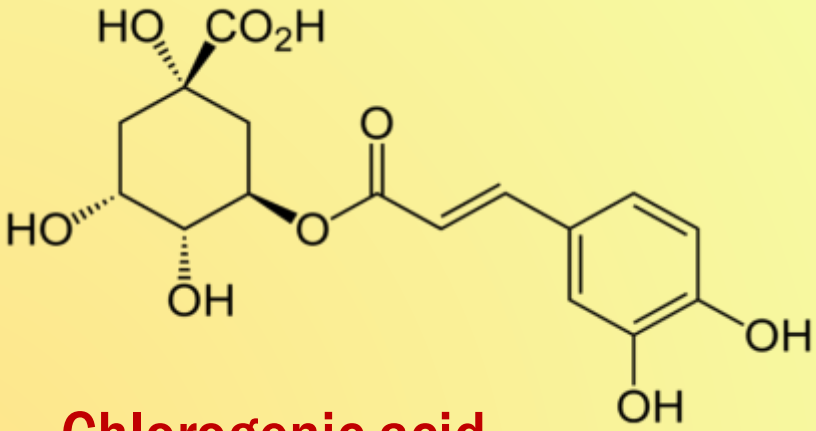
Grape berry composition – Phenolic compounds



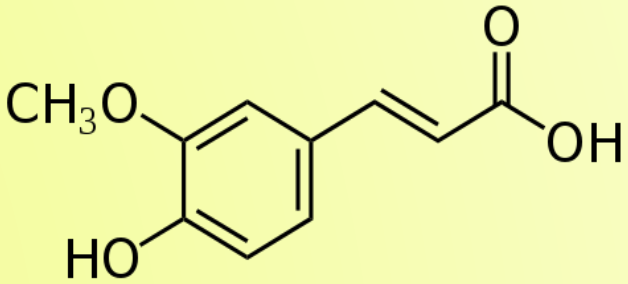
Caffeic acid



p-Cumaric acid



Chlorogenic acid



Ferulic acid

Grape berry composition – Phenolic compounds

Phenolic acids

- These compounds are extracted during vinification and are found in wines at concentrations:
- 100-200 mg/L in red wines
- 10-20 mg/L in white wines
- They are relatively colorless and odorless compounds but are precursors of volatile alcohols by the action of microorganisms (*Bretanomyces*, bacteria, etc.)
- They are found in various parts of the grape mainly in the form of esters but also in free form due to slow hydrolysis reactions

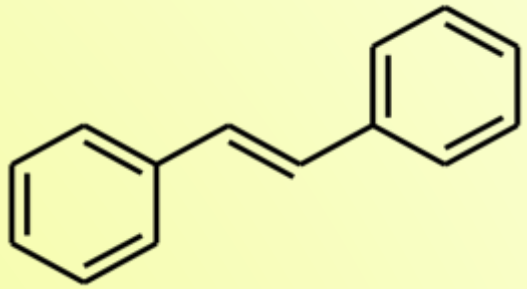
Viticulture – Grape chemistry

Grape berry composition – Phenolic compounds

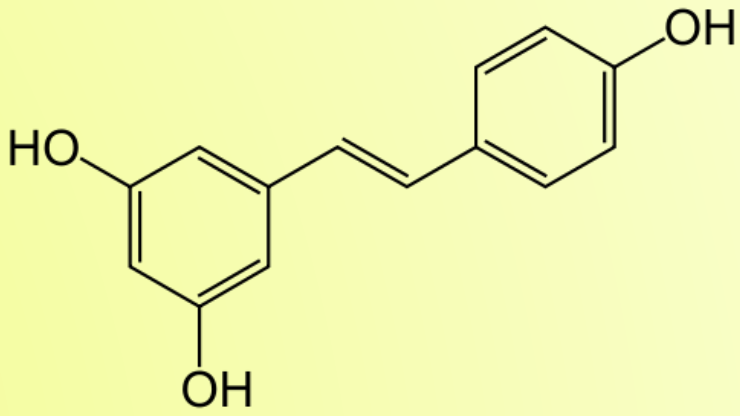
Stilbenes

Complex phenols (stilbene derivatives) found in grapes and pass into wine during winemaking

- ✓ Resveratrol is produced from the vine plant in response to fungal infections (*Botrytis cinerea, Plasmopara viticola*-downy mildew) or by ultraviolet radiation
- ✓ It is found in red wines at 1-3 mg/L
- ✓ It has a beneficial effect on human health
- ✓ Other molecules with similar structures are called Viniferins



Stilbene (E-, trans-)



3,5,4′ -trihydroxy-stilbene (resveratrol)

Viticulture – Grape chemistry

Grape berry composition – Phenolic compounds

Flavonoids

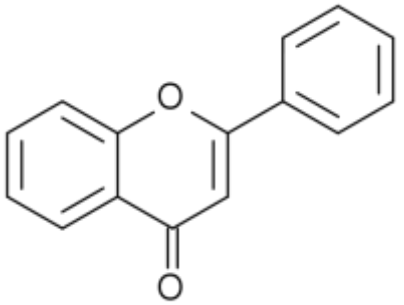
- Flavonoids (latin "*flavus* = yellow) are secondary plant metabolites.

- According to IUPAC they are categorized into:

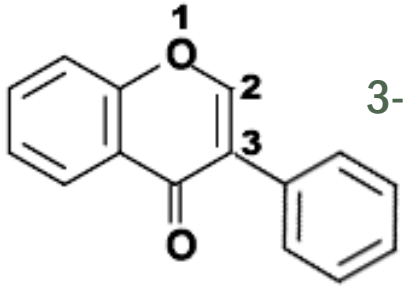
❖ Flavonoids or bioflavonoids

❖ Isoflavonoids; derivatives of 3-phenylchromen-4-one (3-phenyl-1,4-benzopyrone)

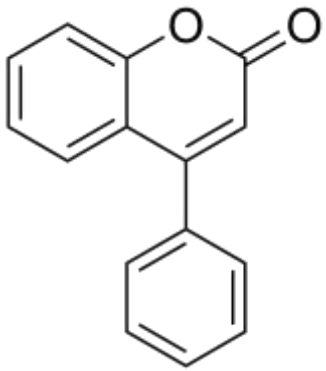
❖ Neoflavonoids; derivatives of 4-phenylcoumarine (4-phenyl-1,2-benzopyrone) structure



Basic flavone structure (2-phenyl-1,4-benzopyrone)



3-phenylchromen-4-one



4-phenylcoumarine

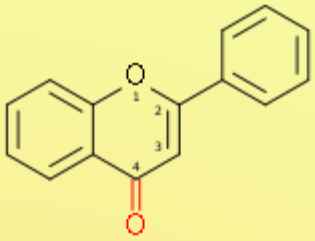
Viticulture – Grape chemistry

Grape berry composition – Phenolic compounds

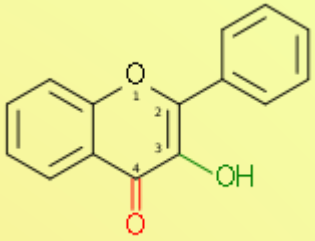
Flavonoids

https://en.wikipedia.org/wiki/Flavonoid

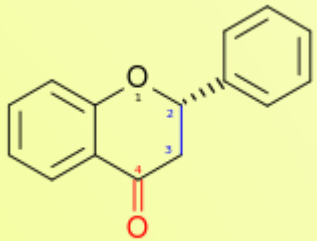
- Their derivatives include reduction of the C2/C3 double bond (flavanones), reduction of the carbonyl group (flavanols), and hydroxyl groups at various positions



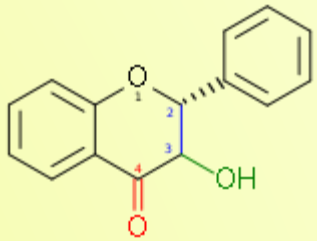
Flavone



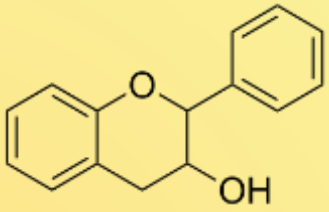
Flavonol
or
3-hydroxyflavone



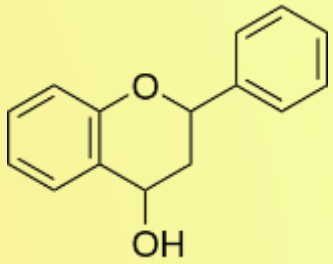
Flavanone



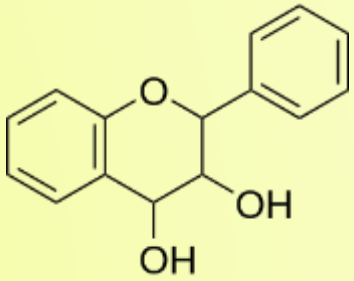
Flavanonol
or
3-Hydroxyflavanone



Flavan-3-ol
(flavanol)



Flavan-4-ol



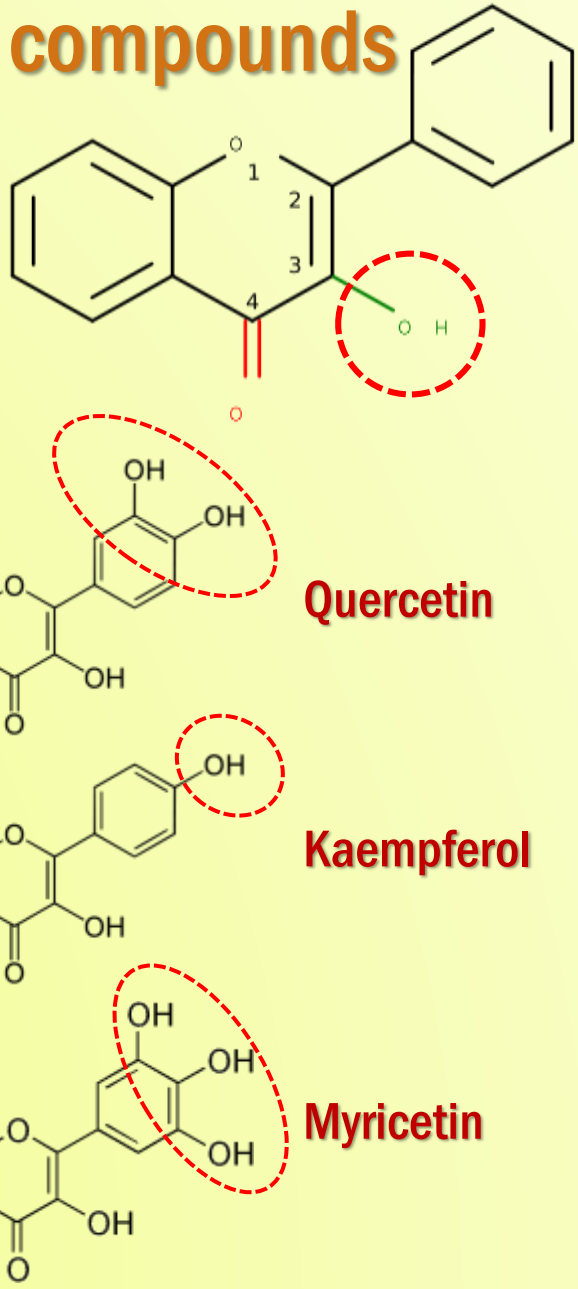
Flavan-3,4-diol
(leucoanzthocyanidin)

Viticulture – Grape chemistry

Grape berry composition – Phenolic compounds

Flavonols (3-hydroxy-flavones) in grapes exist as glucosides, and in wines are found in aglycone forms; **100 mg/L** in red wines and **1-3 mg/L** in white wines:

- **Quercetin** [*3,3',4',5,7-pentahydroxy-2-phenylchromen-4-one*]
- **Kaempferol** [3,4',5,7-tetrahydroxy-2-phenylchromen-4-one]
- **Myricetin** [3,3',4',5',5,7-hexahydroxy-2-phenylchromen-4-one)



Viticulture – Grape chemistry

Grape berry composition – Phenolic compounds

Anthocyans

- Colored pigments of the grape skins and rarely of the flesh (red, purple, blue, depending on the pH value)
- They are glycosides of anthocyanidins (at positions 3, 5)
- Grapes & wines usually contain 5 different anthocyanidin molecules:

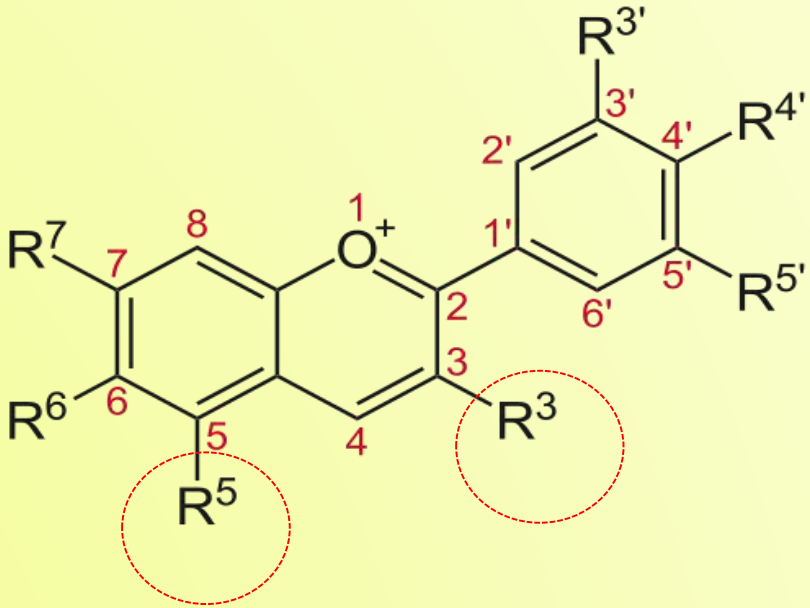
✓ Delphinidin

✓ Petunidin

✓ Malvidin

✓ Peonidin

✓ Cyanidin



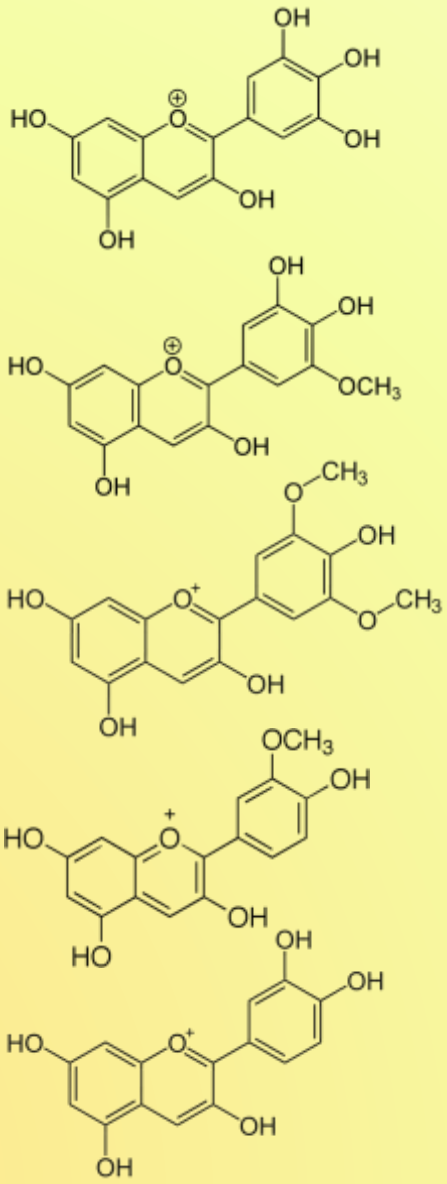
Anthocyanidine (aglycone form)

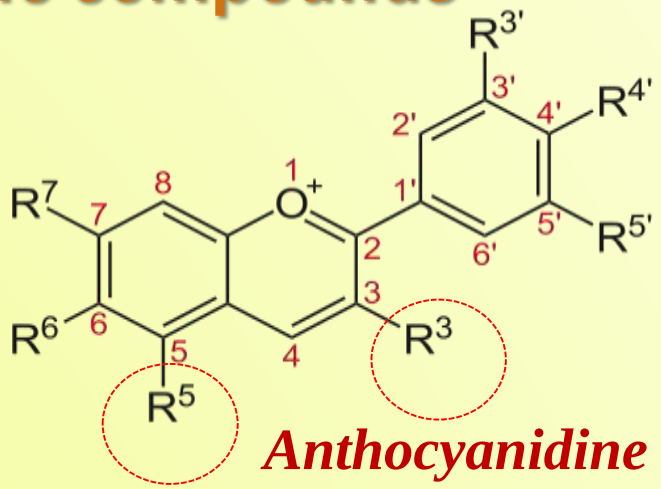
Viticulture – Grape chemistry

Grape berry composition – Phenolic compounds

Anthocyans

- ✓ Delphinidin
- ✓ Petunidin
- ✓ Malvidin
- ✓ Peonidin
- ✓ Cyanidin



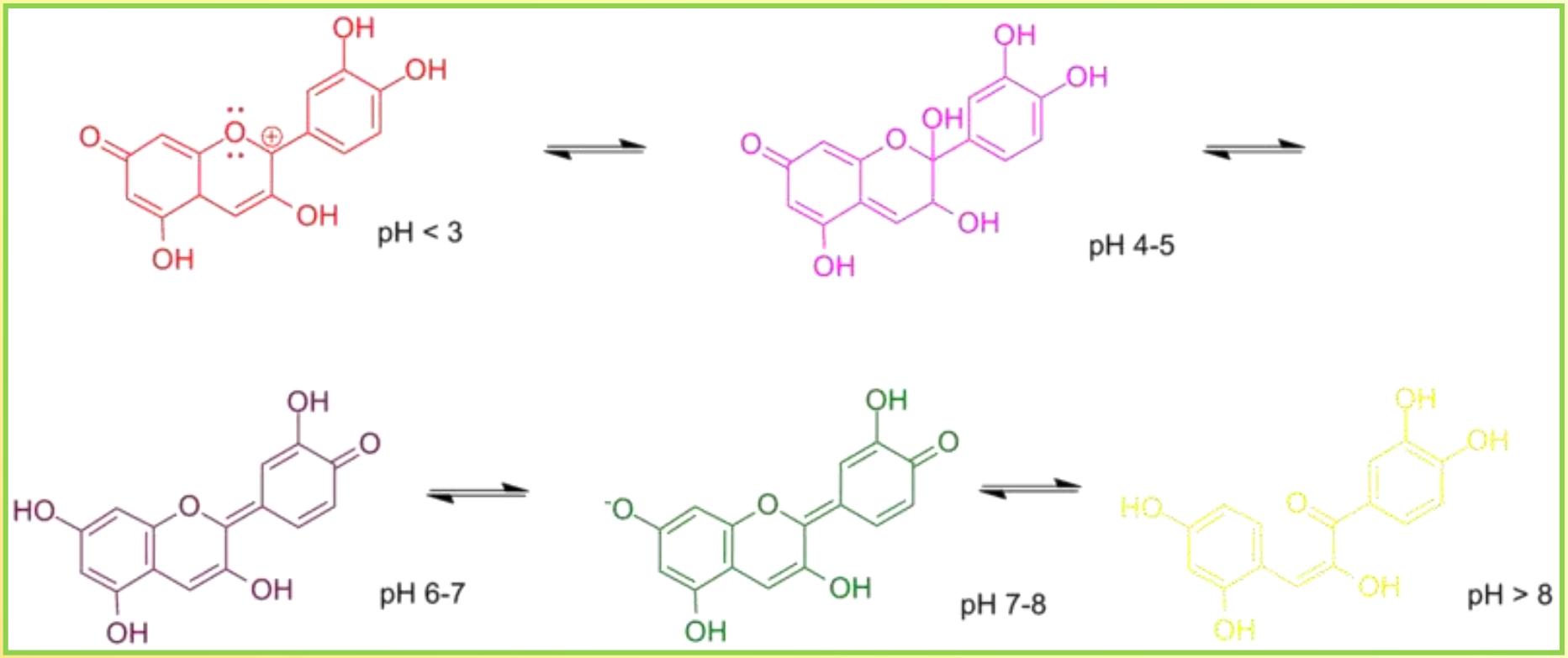


Anthocyanidine (aglycone form)

Anthocyan	$R^{3'}$	$R^{4'}$	$R^{5'}$	R^{3}	R^{5}	R^{6}	R^{7}
Cyanidin	OH	OH	H	OH	OH	H	OH
Delphinidin	OH	OH	OH	OH	OH	H	OH
Malvidin	OCH_3	OH	OCH_3	OH	OH	H	OH
Peonidin	OCH_3	OH	H	OH	OH	H	OH
Petunidin	OH	OH	OCH_3	OH	OH	H	OH

Grape berry composition – Phenolic compounds

Color & stability of anthocyanidins depending on pH

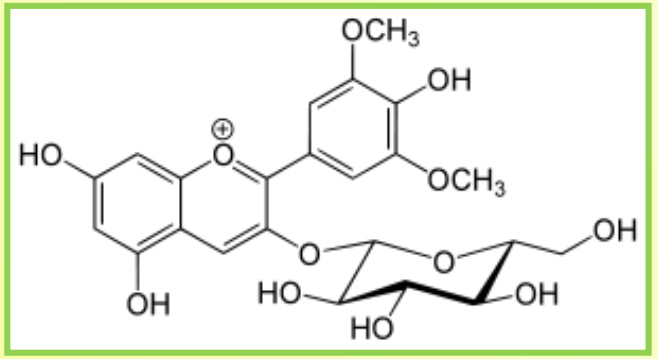


Viticulture – Grape chemistry

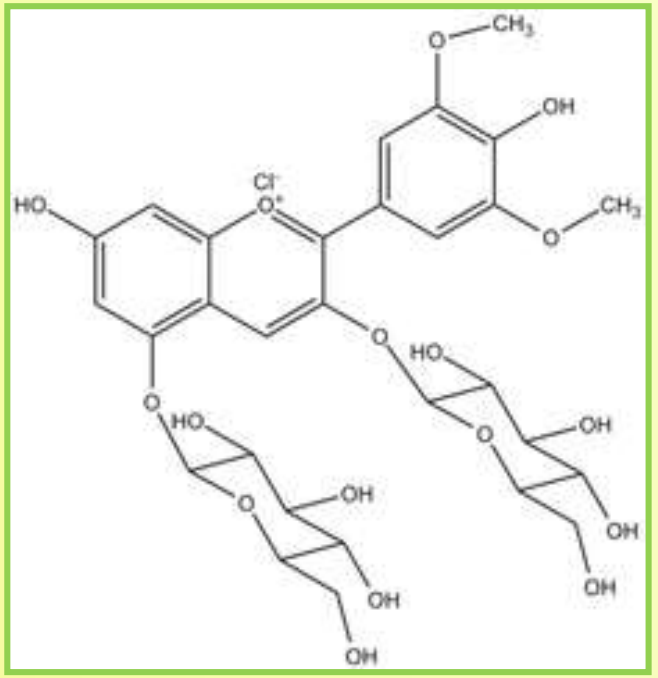
Grape berry composition – Phenolic compounds

Anthocyans

- In *Vitis vinifera* 3-monoglycosides have been identified, while diglycosides can be found in other species (*V. riparia*, *V. rupestris*)
- Malvidin predominates in most red varieties and is the basis of red wine color (100 mg/L in Pinot Noir, 1500 mg/L in Cabernet Sauvignon)
- Over the years their color fades, but they bind with tannins to form undefined structure compounds with stable colors



3-glycoside of malvidin



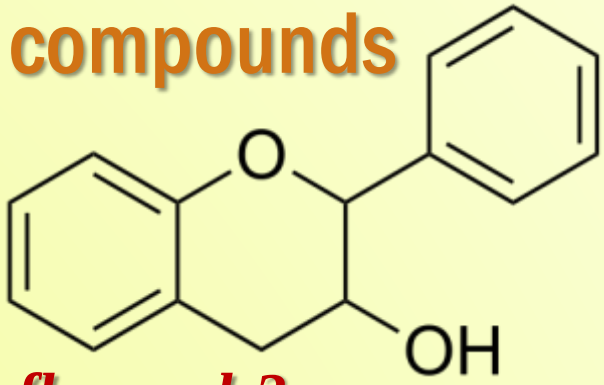
3,5-diglycoside of malvidin

Viticulture – Grape chemistry

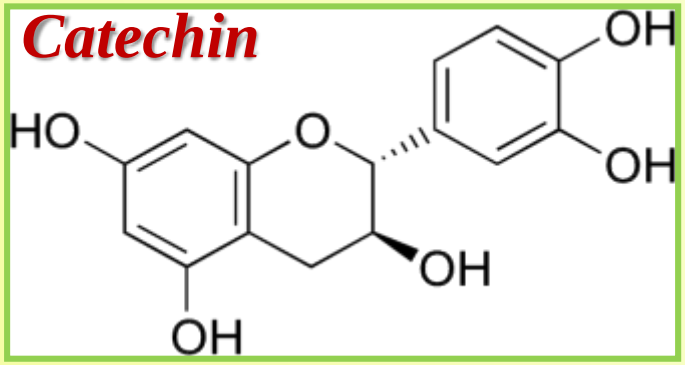
Grape berry composition – Phenolic compounds

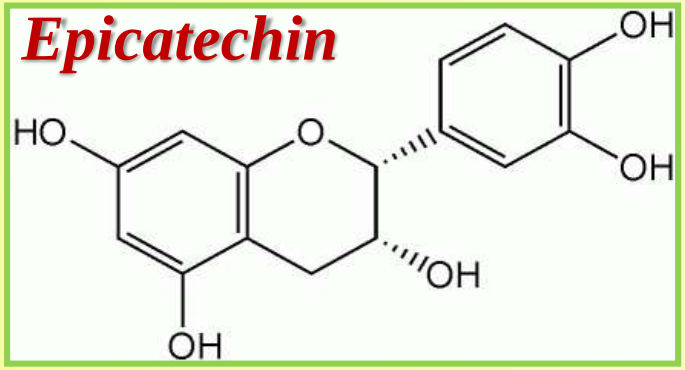
Catechins and their polymers (procyanidins)

- Catechins are hydroxylated derivatives of flavanol-3
- When heated in an acidic environment they form dark brown compounds
- These derivatives (procyanidins) when cleaved provide catechin, epicatechin and cyanidine
- In grapes there are condensed molecules up to 4 units - In wines they have smaller MWs (dimers & trimers)
- Aging results in polymers of larger MWs (2000-3000) corresponding to condensed tannins



flavanol-3



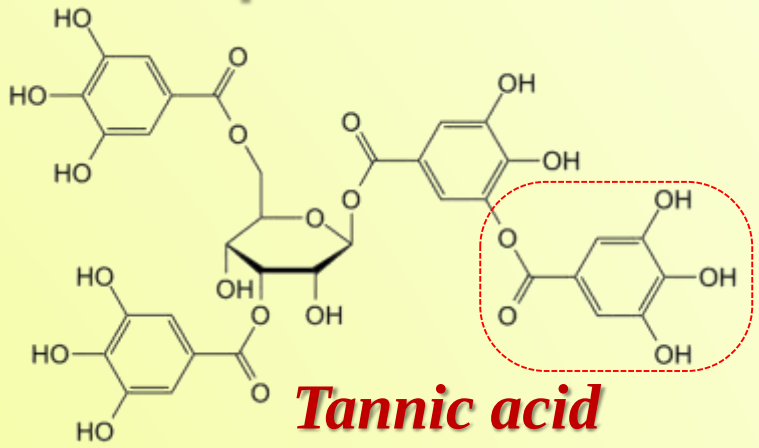


Viticulture – Grape chemistry

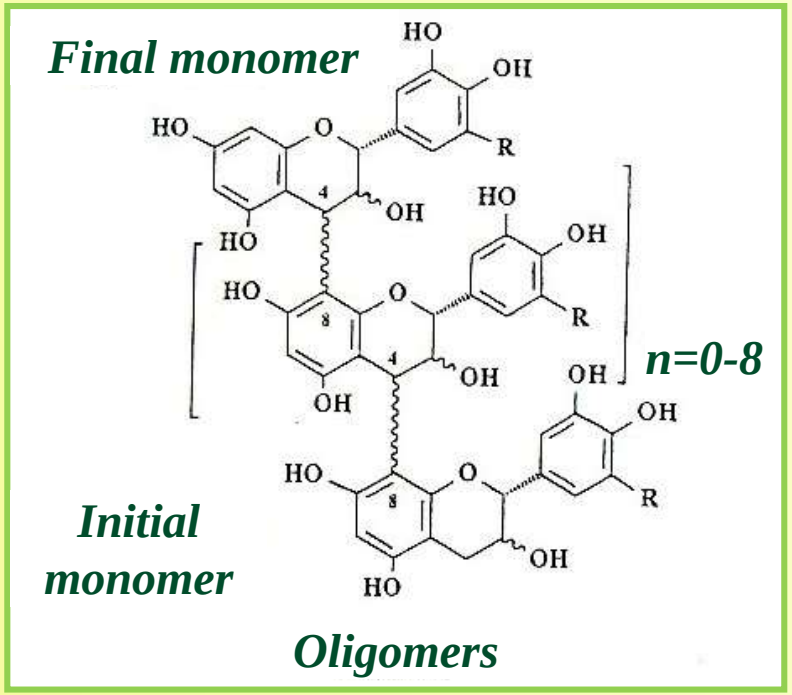
Grape berry composition – Phenolic compounds

Tannins

- **Tannins** are agglomerates of phenolics produced by polymerization – they are present in all parts of the grape (MW 600-3500)
- They form stable compounds with proteins and polysaccharides
- **Hydrolysable tannins**: Gallotannins and Ellagitannins) (they decompose into gallic & ellagic acid respectively) - not present in grapes)



Tannic acid



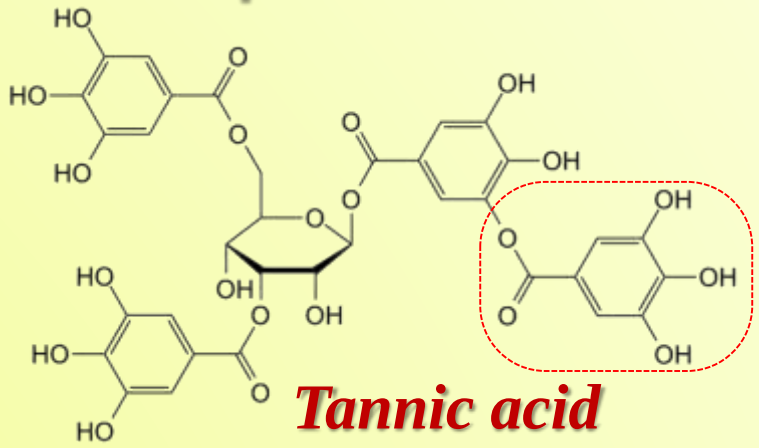
Proanthocyanidins

Viticulture – Grape chemistry

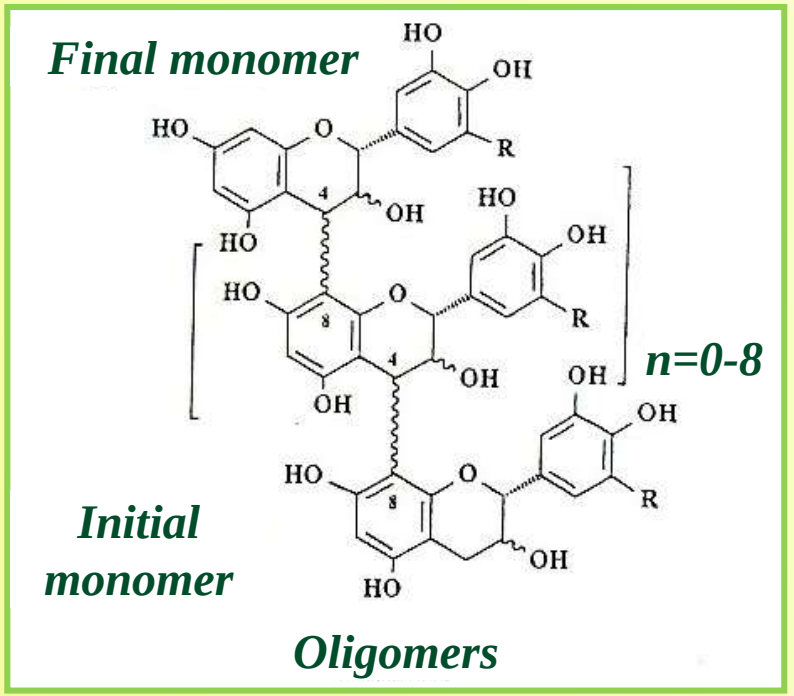
Grape berry composition – Phenolic compounds

Tannins

- Non-hydrolyzable tannins: catechin tannins
- They play an important role in the aging of wines in oak barrels due to their antioxidant properties and the effect on taste
- In wines, depending on the variety and the way of vinification 1-4 g/L are found



Tannic acid



Proanthocyanidins

Viticulture – Grape chemistry

Grape berry composition – Phenolic compounds

Properties of phenolic compounds

- **Grape skin color:** mainly anthocyanins and flavonoids, and secondarily carotenoids (in white varieties). The color depends on the variety, the concentration of sugars, the pH, etc.

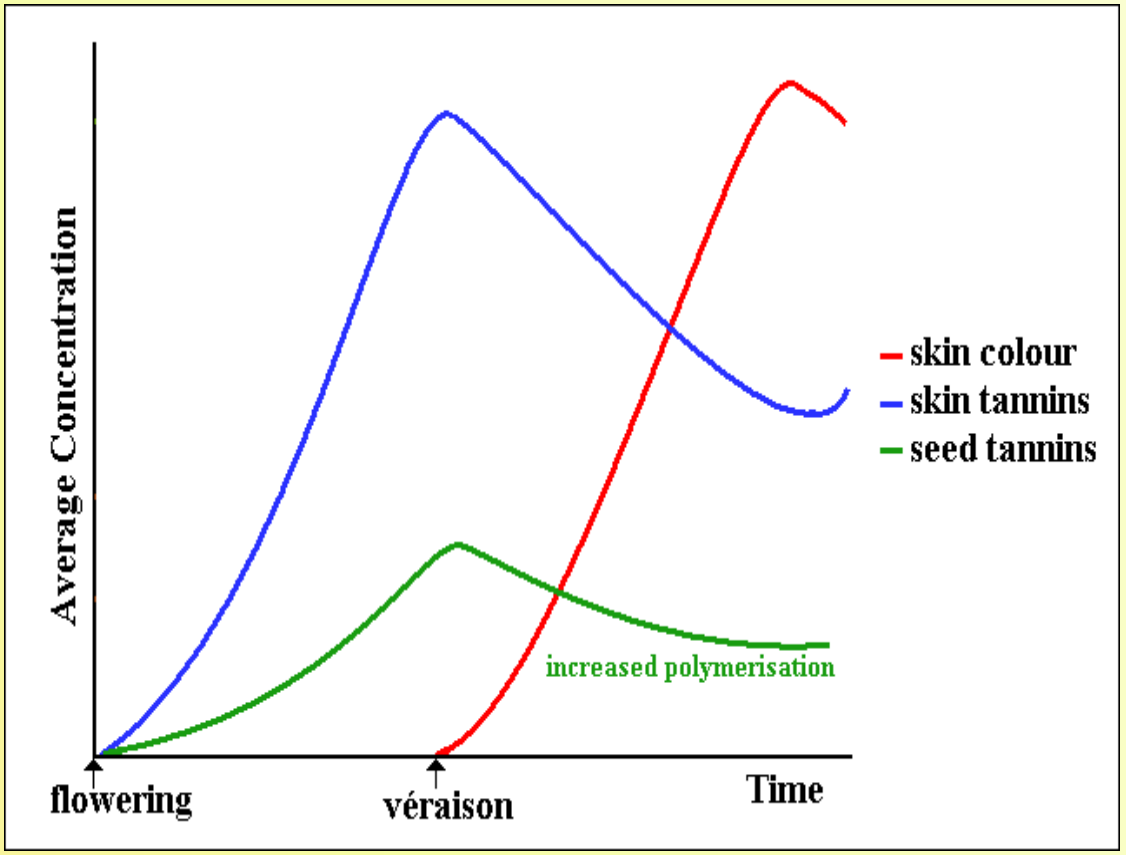
- **Leaf color:** **European varieties**: at the end of the germination period the color of the leaves of white varieties turns yellow, while those of red varieties first turn yellow and then red. **American varieties**: the leaves always turn yellow (with a few exceptions)

Viticulture – Grape chemistry

Grape berry composition – Phenolic compounds

Development of phenolics during maturation

- During véraison/maturation the phenolic compounds of the skins increase.
- Anthocyans appear during véraison and accumulate during maturation, while during over-maturation they decompose. Their concentration depends on the growing conditions and climate.
- Tannins increase accordingly

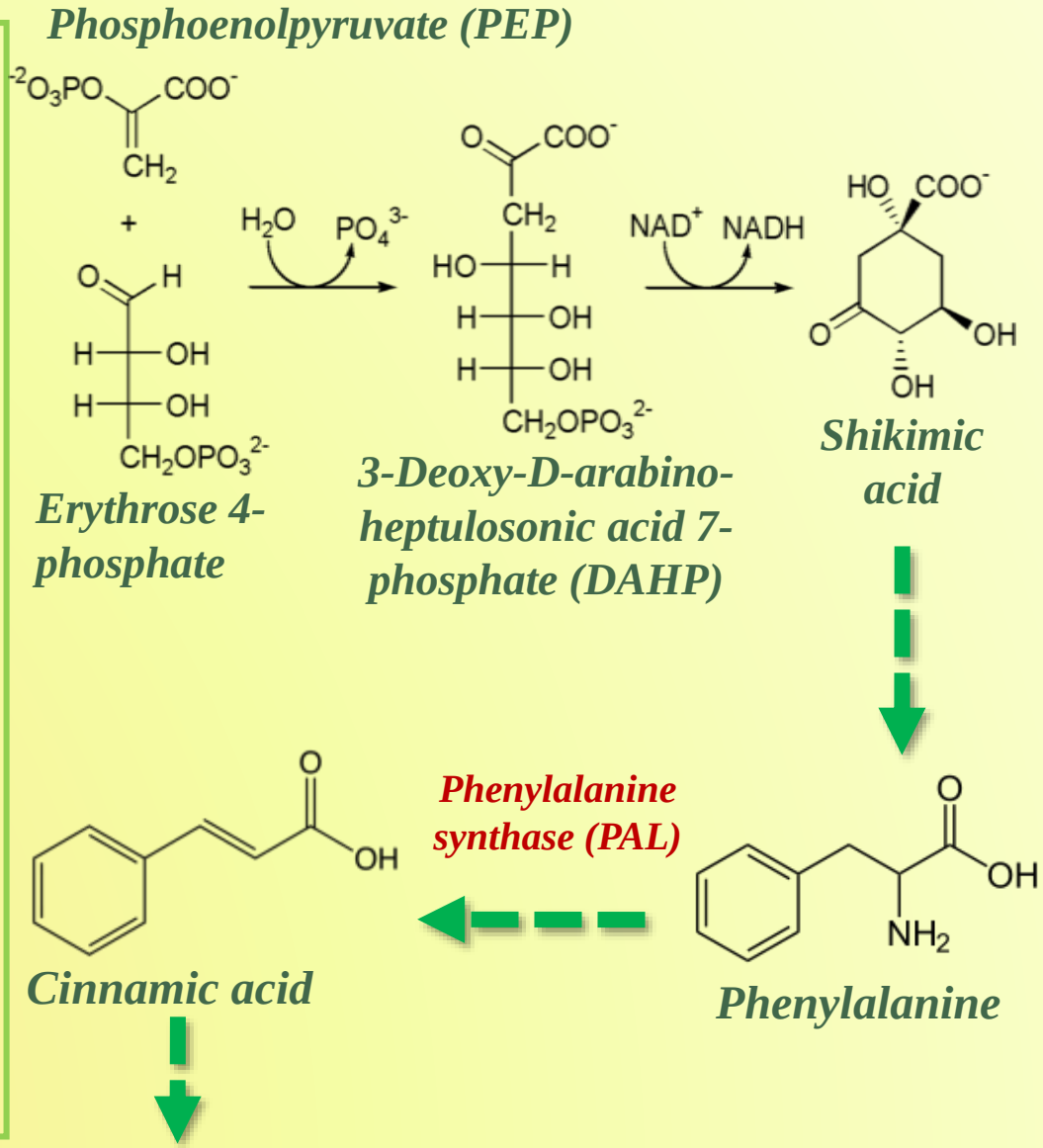


- In the grape seeds, the concentration of tannins decreases at véraison and is related to the concentration of anthocyans in the skins

Grape berry composition – Phenolic compounds

Biosynthesis of phenolics

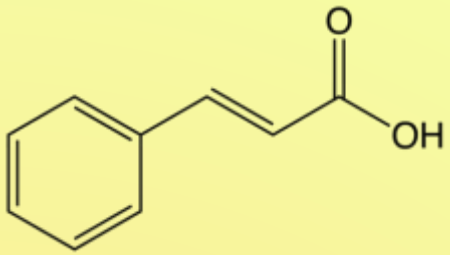
- Phenolic compounds are products of the secondary sugar metabolism
- Benzoic and cinnamic acid derivatives come from the condensation of erythrose 4-phosphate (a derivative of the phosphate pentose pathway) and phosphoenolpyruvate (PEP)
- Another way is through glycolysis and production of malonyl-CoA by condensing 3 acetyl-CoAs and CO_2



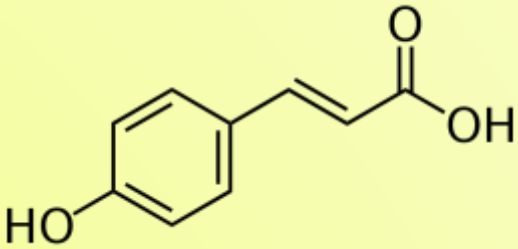
Viticulture – Grape chemistry

Grape berry composition – Phenolic compounds

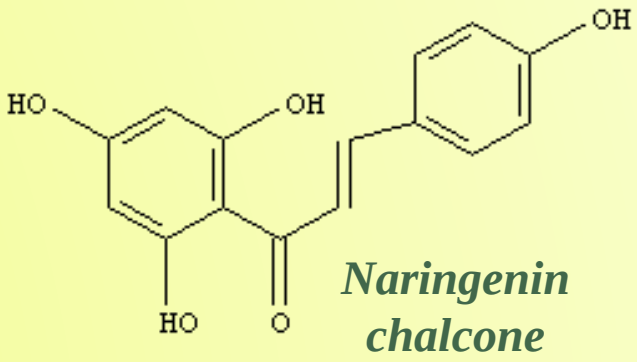
Biosynthesis of phenolics



Cinnamic acid



p-Cumaric acid



Naringenin chalcone

Flavonoids

Anthocyans

Grape berry composition – Phenolic compounds

Factors affecting the phenolics accumulation

- ✓ The competition in the conversion of phenylalanine into proteins or phenolics
- ✓ The sugar content in the grapes
- ✓ Environmental conditions (temperature, sun light, water, nitrogen nutrition, etc.).
  - E.g., the enzyme PAL (*phenylalanine lyase*) is activated by light but a reduction in light intensity has not been found to limit the synthesis of phenolic components.
  - Temperature plays an important role on grape pigmentation (at extreme conditions). Optimal temperature for anthocyanin synthesis is 20°C. Higher temperatures affect indirectly due to the formation of larger grape berries with higher skin weight.
- ✓ Changes in the hormonal balance of the plants: if it promotes plant growth, it reduces the production of phenolics. So does the availability of nitrogen & water in the period that favors the plant growth.

Viticulture – Grape chemistry

Grape berry composition – Aroma compounds

- The aroma of the wine products is the result of hundreds of ingredients with very small concentrations (from mg/L to ng/L)
- The perception threshold value of these components varies widely
- The complexity of wine aroma is such that it is very difficult to quantify/qualify, and is related to:
  - ✓ The metabolism of the vine plant which depends on the variety, the environment & the cultivation practice
  - ✓ The biochemical changes before alcoholic fermentation (oxidation, hydrolysis, etc.)
  - ✓ The alcoholic fermentation (yeast metabolism)
  - ✓ Chemical changes after fermentation (maturation/ageing)
  - ✓ The ageing vessels (extracted wood components)

Viticulture – Grape chemistry

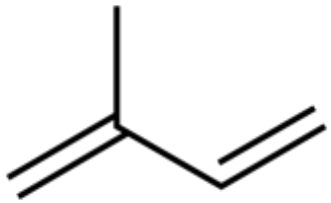
Grape berry composition – Aroma compounds

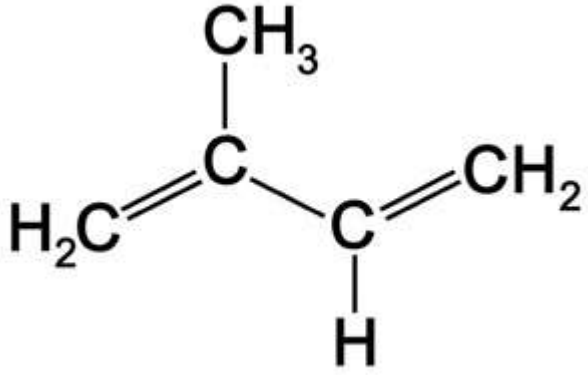
- Substances derived from the grape fruits and reflect the characteristics of the specific variety - they are responsible for the "varietal aroma" of the wine product (especially in Muscats)
- The most characteristic compounds that contribute to the Muscat character are terpene alcohols (monoterpenes, 10 C, 2 isoprene units):

Isoprene:

2-methyl-1,3-butanediene

C_5H_8

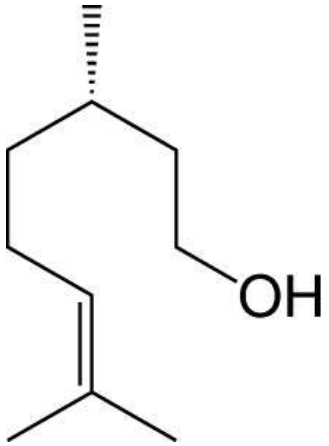




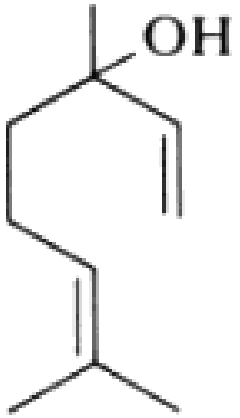
Viticulture – Grape chemistry

Grape berry composition – Aroma compounds

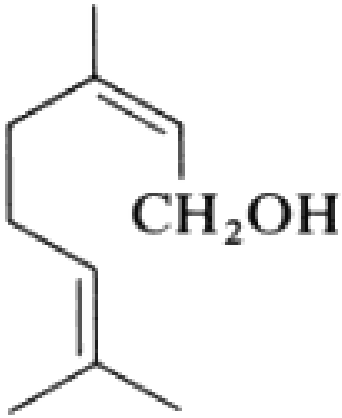
Terpene alcohols



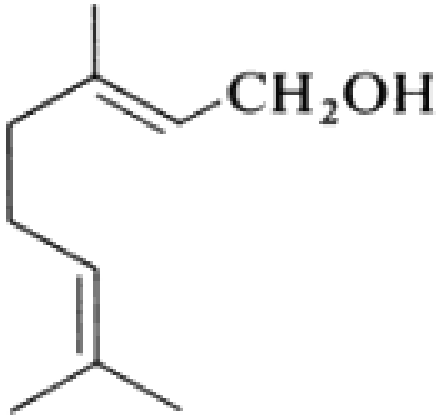
β-Cirtronellol
3,7-Dimethyl-oct-6-en-1-ol



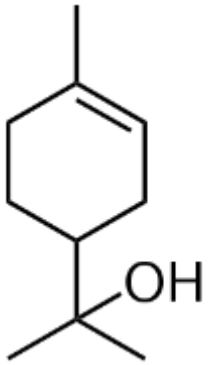
Linallool
3,7-Dimethyl-octa-1,6-dien-3-ol



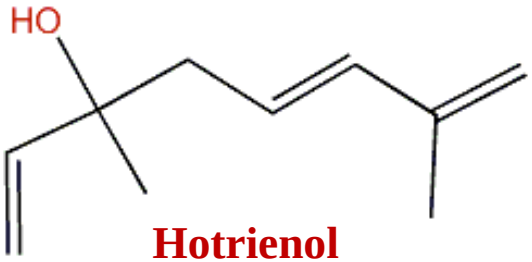
Nerol
(2Z)-3,7-Dimethyl-octa-2,6-dien-1-ol
(cis isomer)



Geraniol
(2E)-3,7-Dimethyl-octa-2,6-dien-1-ol
(trans isomer)



α-Terpineol
2-(4-Methyl-cyclohex-3-en-1-yl)propan-2-ol
(*p*-Menth-1-en-8-ol)



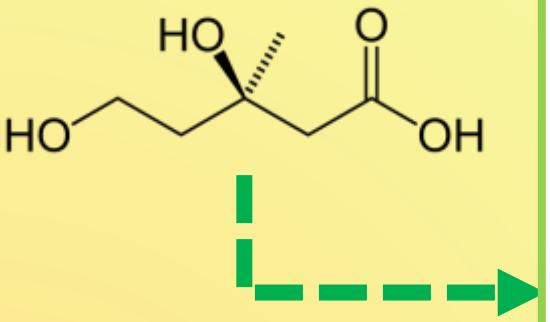
Hotrienol
3,5-dimethyl-octa-1,5,7-trien-3-ol

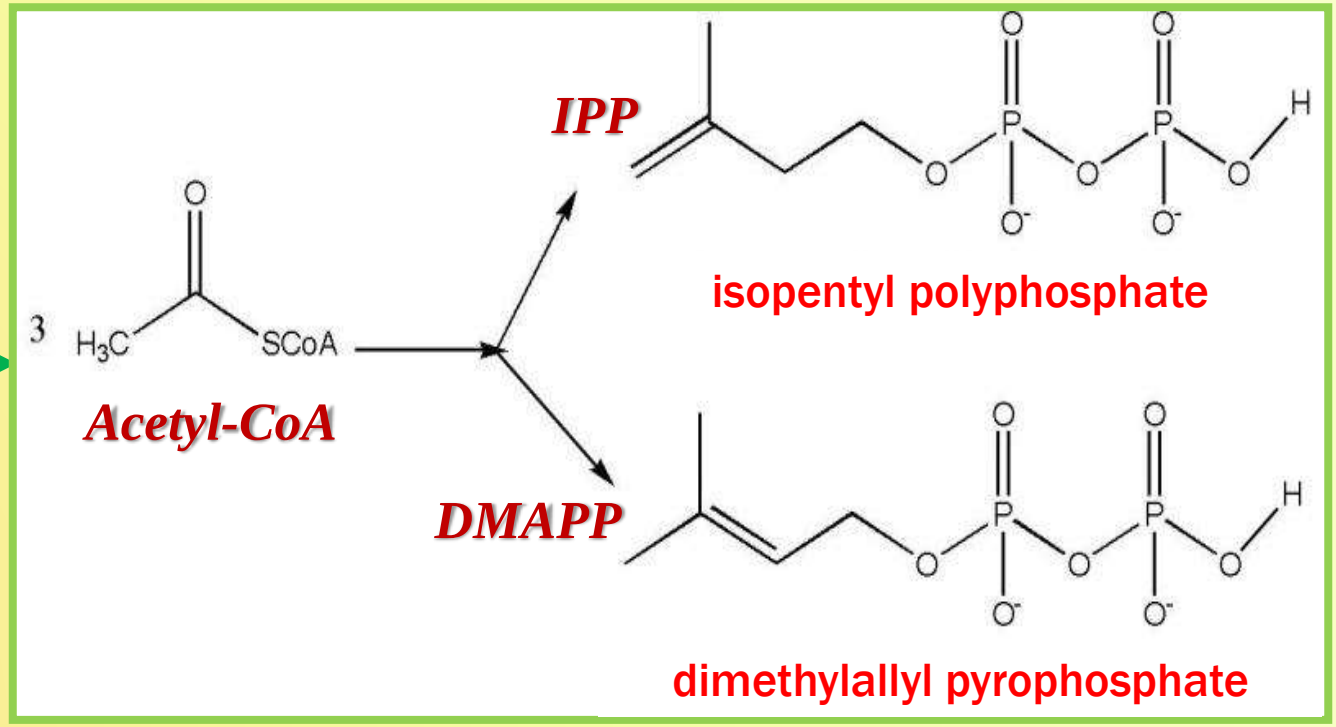
Viticulture – Grape chemistry

Grape berry composition – Aroma compounds

Precursor of terpenes (cyclic and acyclic) is mevalonic acid which is a precursor of Acetyl-CoA and subsequently the phosphorylated derivatives of hemiterpene (isoprene): isopentyl polyphosphate (IPP) & dimethylallyl pyrophosphate (DMAPP)

Mevalonic acid
(3R)-3,5-Dihydroxy-3-methyl-pentanoic acid

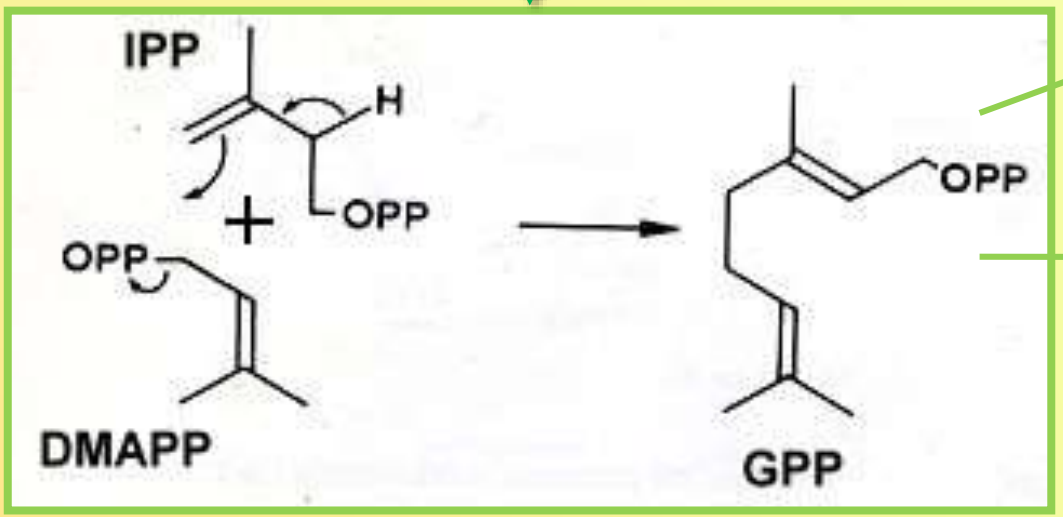




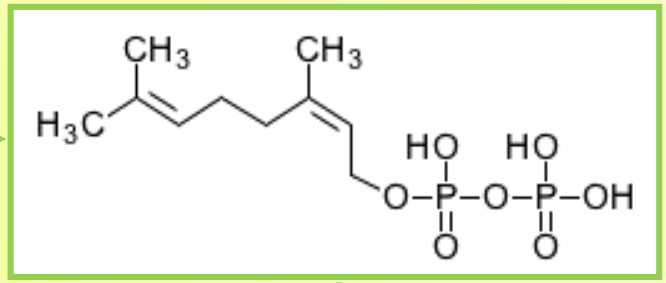
Viticulture – Grape chemistry

Grape berry composition – Aroma compounds

Terpenoids are produced by condensing isopentelyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP) into geranyl pyrophosphate (GPP)



Non-cyclic terpenes

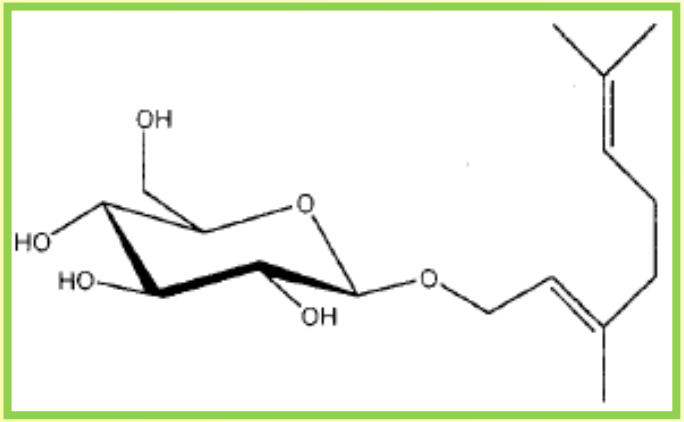


Cyclic terpenes

Viticulture – Grape chemistry

Grape berry composition – glycosides of volatile terpenes

- Glycosides of volatile terpenols are odorless non-volatile compounds (compounds combined with sugars such as glucose, arabinose and rhamnose)
- They are contained in all varieties and to a greater extent in Muscats, and in higher concentrations in the grape skins than other parts of the grape
- The relative proportion of free and bound forms (glycosides) depends on the variety
- Glycosylated forms are more soluble and move more easily between different parts of the plant
- Free terpenols are released from their glycoside forms by the action of enzymes (*β-glucosidases*)



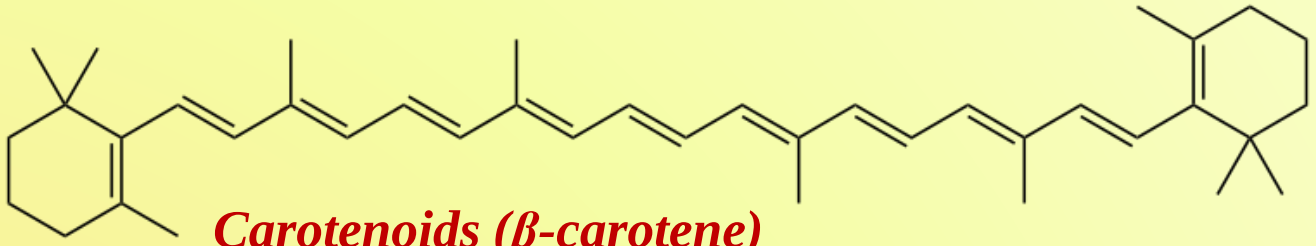
Geraniol glycoside

- The grape must of Muscat of Alexandria contains more bound than free terpenols, while the skins contain equal amounts
- In White Muscat the proportions of the two forms are the same

Viticulture – Grape chemistry

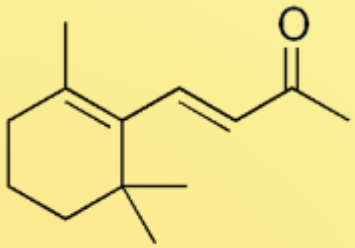
Grape berry composition – norisoprenoids

Enzymatic and oxidative breakdown of carotenoids into 13 C compounds are called Norisoprenoids (they have aromas of tropical fruits and flowers)



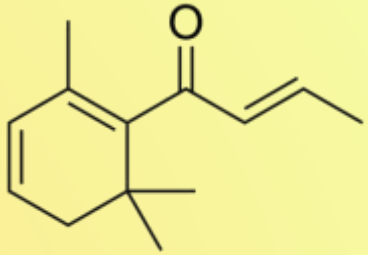
Carotenoids (β-carotene)

Norispoprenoids



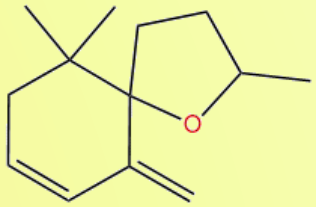
β-Ionone

(3*E*)-4-(2,6,6-Trimethylcyclohex-1-en-1-yl)but-3-en-2-one



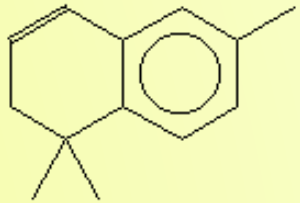
β-Damascenone

(*E*)-1-(2,6,6-Trimethyl-1-cyclohexa-1,3-dienyl)but-2-en-1-one



Vitispirane

2,10,10-trimethyl-6-methylidene-1-oxaspiro[4.5]dec-7-ene



TDN

1,1,6,-trimethyl-1,2-dihydronapthalene

Viticulture – Grape chemistry

Grape berry composition – norisoprenoids

- **β-Ionone:**

✓Violet/wood/raspberry aromas - Perception threshold 7 ng/L in aqueous solution.

✓Small contribution to the aroma of white wines but very large to red and muscat wines.

- **β-Damascenone:**

✓Aroma of tropical fruits/baked apple/quince/flowers - Perception threshold 2 ng/L in aqueous solution.

✓In sweet white wines the level of perception is ~4.5 µg/L and in red with different levels of perception and concentrations up to 50-4000 ng/L, depending on the type of wine.

✓Large quantities are found in sweet Muscat wines.

Viticulture – Grape chemistry

Grape berry composition – norisoprenoids

- **Vitispirane:**

✓ Aroma of camphor/eucalyptus. Perception threshold 800 µg/L in aqueous solution.

✓ Found at 4 stereoisomeric structures with different aromas

- **TDN:**

✓ Aroma of kerosene/petroleum. Perception threshold 20 µg/L in aqueous solution.

✓ Typical aroma of aged Riesling (up to 200 µg/L) and generally undesirable for wines.

✓ It is synthesized in larger quantities in conditions of nitrogen deficiency.

Viticulture – Grape chemistry

Grape berry composition – other aroma compounds

- **Methoxypyrazines**:

✓They are produced from the metabolism of amino acids

✓They have peppery aromas

✓*(Earthy, pepper, potato, pea)*

R= $CH(CH_3)_2$:
2-methoxy-3-isopropylpyrazine

R= $CH_2CH(CH_3)_2$:
2-methoxy-3-isobutylpyrazine

R= $CH(CH_3)CH_2CH_3$:
2-methoxy-3-butylpyrazine

Viticulture – Grape chemistry

Grape berry composition – other aroma compounds

- **Thiols**:

✓They are found in complexes with cysteine

✓Mainly found in **Sauvignon blanc** and are release during alcoholic fermentation by the action of ***lyase*** enzyme

✓CH_3-CH_2-CH_2-CH(SH)-CH_2OH

3-mercapto-1-hexanol

Sulfurous, fruity, meaty, coffee, roasted, coffee

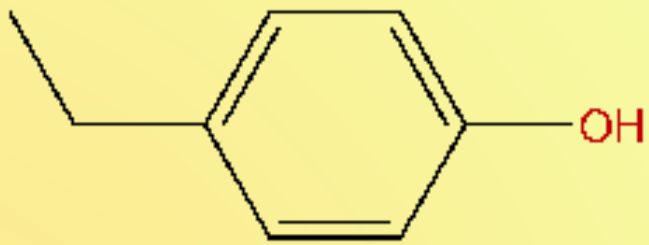
Viticulture – Grape chemistry

Grape berry composition – other aroma compounds

- **Volatile phenols**:

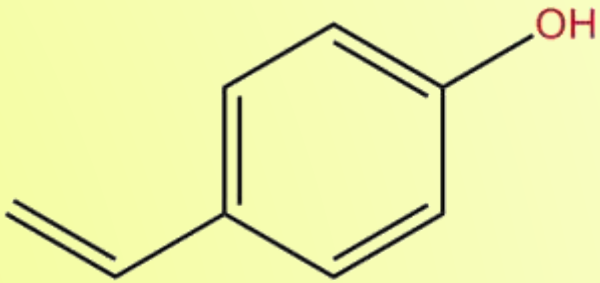
✓ Ethylphenol (red wines)

✓ Vinylphenol (white wines)



Ethylphenol

Chemical, phenolic, medicinal, sweet, musty, meaty



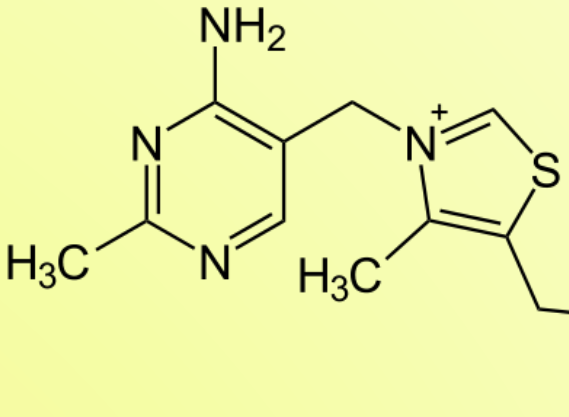
Vinylphenol

Chemical, phenolic, medicinal, sweet, musty, meaty

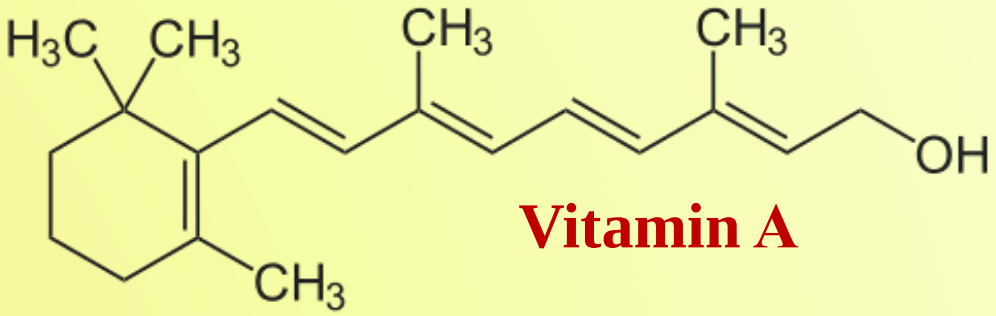
Viticulture – Grape chemistry

Grape berry composition – vitamins

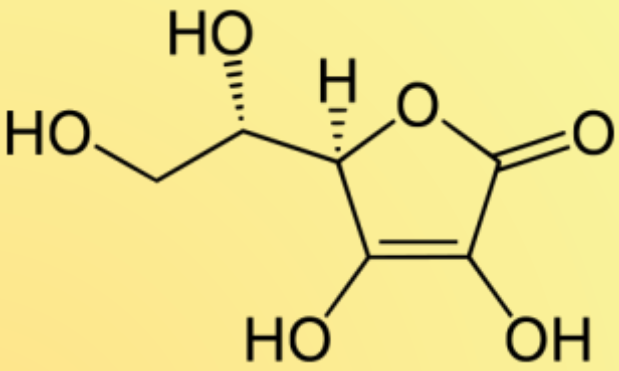
- **Vitamin C (ascorbic acid)**
- **Vitamins B**
- **Vitamin A**
- **Vitamin E**



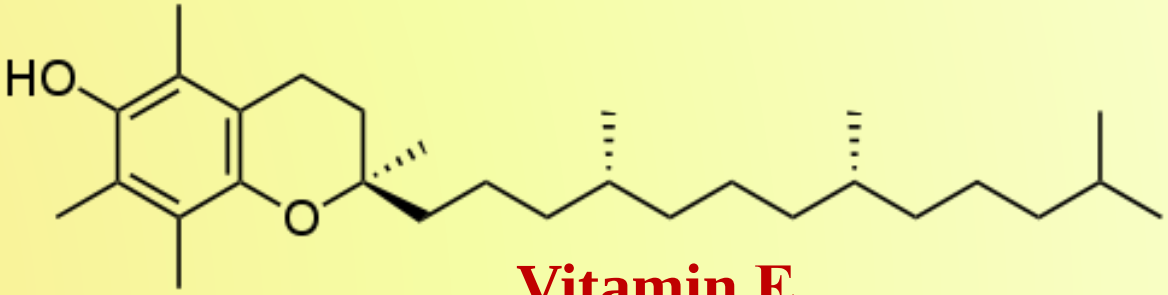
Vitamin B1 (thiamin)



Vitamin A



Vitamin C



Vitamin E

Αμπελουργία – Χημεία της Αμπέλου

Final exam question styles

1. **Provide a brief summary (one page at most) of the coursework you did in the laboratory.**
2. **What do you know about the “xxx" variety (from those discussed in the course) and the wine produced from it?**
3. **Role / morphology and main functions of the root system and leaves of the vine.**
4. **Stages of grape maturation (biological and technological maturity).**
5. **Diseases and nutrient deficiencies of the vine (causes and how they are treated).**
6. **Climates and microclimates that favor the cultivation of the vine and affect the maturation of grapes, their composition, the harvest period, and the quality of the wine.**
7. **Sugars and organic acids that predominate in the composition of unripe and mature grapes.**

Αμπελουργία – Χημεία της Αμπέλου

Final exam question styles

1. **Forms of nitrogen assimilated by the vine.**
2. **Origin of chemical compounds that compose the final aroma of wine?**
3. **Organic viticulture and wine making.**
4. **MATCH (with arrows) the chemical compounds (e.g. β-citronellol, malvidin, resveratrol, etc.) with the corresponding categories of compounds to which the compounds belong (e.g. anthocyanidin, polyamine, flavonoid, stilbene, etc.).**
5. **MENTION CATEGORIES of chemical compounds that are present in grapes and are important (and why) for the aroma of wines.**
6. **RECOGNIZE the structures of several compounds: e.g. catechin, resveratrol, phenolic acids, terpenes, flavonoids, etc.**

Thank u!